University of Alberta

Evolution of skeletal muscle and adipose tissue loss in advanced cancer by dual energy and CT imaging

by

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Dedicated with love to:

My loving husband:
Mohammad Reza

And to

My two precious futures:
Maryam and Amirreza

ABSTRACT

Cachexia, a well-known complication of many life-threatening diseases, is seen in most patients with advanced cancer. Cachexia is a syndrome that is mainly characterized by progressive weight loss and hyper-catabolism, which results in loss of body tissue such as skeletal muscle and adipose tissue. The study objectives were to evaluate the intensity of muscle and adipose tissue wasting in a cancer population, to determine the role of gender in cachexia prevalence, and to investigate the relationship between nutritional intake and body composition changes in cancer patients. Body composition was measured using two approaches: A retrospective study using CT (Computerized Tomography) scans and a prospective studying using DEXA (Dual Energy X-ray Absoptiometry). The results showed that skeletal muscle is the main tissue that is wasted during the progression of cancer. Adipose tissue loss and gain were almost equally prevalent. More male patients lost muscle and SC (Subcutaneous) adipose tissue than females. With respect to age, the main difference was seen in VAT (Visceral Adipose Tissue). The older group lost VAT with a greater rate of change than the younger group. Evaluating nutritional intake in cancer patients showed that the average energy intake in this study population was slightly lower than the general population. In general, the results of this study confirmed that the behaviour of body composition change in people with cancer varies among individuals and does not follow a unique pattern.

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List of Abbreviations

A.N: Anorexia-Nervosa
ACB: Alberta Cancer Board
AIDS: Acquired Immune Deficiency Syndrome
AMA: Arm Muscle Area
AT: Adipose Tissue
ATP: Adenosine Triphosphate
BCM: Body Cell Mass
BIA: Bioimpedance Analysis
BMC: Bone Mineral Content
BMD: Bone Mineral Density
BMI: Body Mass Index
BW: Body Weight
CCI: Cross Cancer Institute
CHF: Chronic Heart Failure
COPD: Chronic Obstructive Pulmonary Disease
C-RP: C-Reactive Protein
CT: Computerized Tomography
DEXA: Dual Energy X-ray Absoptiometry
DHEA: Dehydroepiandrosterone
DICOM: Digital Imaging and Communication In Medicine
DPA: Dual Photon Absorptiometry
ECOG: Eastern Cooperative Oncology Group
EPA: Eicosapentaenoic Acid
EDL: Extensor Digitorum Longus
FFA: Free Fatty Acid
FFM: Fat Free Mass
FM: Fat Mass
GLT: Grey Level Image

HIV: Human Immunodeficiency Virus

HSL: Hormone-Sensitive Lipase

HU: Hounsfield Unit

IL: Interleukin

INF: Interferon

KeV: Kiloelectron volts

Kvp: Kilovolt peak

LLA: Lipoprotein Lipase enzyme Activity

LMF: Lipid Mobilizing Factor

MA: Megestrol Acetate

mA: Milliampere

MAC: Mid Arm Circumference

MHC: Myosin Heavy Chain

MR: Milliroentgens

MRI: Magnetic Resonance Imaging

mRNA: messenger RNA

N/V: Nausea and Vomiting

NSCLC: Non Small Cell Lung Cancer

PACS: Picture Archiving and Communication Systems

PG-SGA: Patient Generated-Subjective Global Assessment

PIF: Proteolysis Inducing Factor

SC: Subcutaneous

SGA: Subjective Global Assessment

SM: Skeletal Muscle

TBK: Total Body potasium

TSF: Triceps Skinfold Thickness

VAT: Visceral Adipose Tissue

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List of Definitions

Attenuation

Weakening in force or intensity. The property of something that has been weakened or reduced in thickness or density

Bone mineral content (BMC)

Bone mass expressed as mineral per centimetre of bone. It is used to assess amount of bone accumulation prior to cessation of growth.

Bone mineral density (BMD)

The compactness of bone; mass of one per unit of area (g/cm^2). Used to assess bone post development. Most accurately measured by DEXA.

KeV

Kiloelectron volts, a unit of effective mean X-ray tube voltage in diagnostic radiography. In particle physics, a unit of energy that is commonly used is the electron-volt (eV).

$1\ eV = 1.6\ 10^{-19}$ Joules

Kvp

The peak accelerating voltage applied in an x-ray tube between the cathode and anode

Pixel

The smallest resolvable rectangular area of an image, either on a screen or stored in memory.

Reliability

Reproducibility or repeatability of results using a particular method

Tungsten

Rare element of the chromium group found in certain minerals, such as wolfram and scheelite, and isolated as a heavy steel-grey metal, which is very hard and infusible. It has both acid and basic properties. When alloyed in small quantities with steel, it greatly increases its hardness.

Validity

Refers to whether the method measures what it is supposed to measure.

Chapter One

Introduction

A. Introduction and Rationale

Cancer is a growing health problem worldwide. It has been estimated that 145,500 new cases of cancer and 68,300 deaths due to cancer will occur in Canada in 2004. Based on the current incidence, 43% of men and 38% of women will face cancer during their lifetime (1).

Many patients with advanced cancer experience multiple physical and psychological complications, which result in a burden to the health system. Although pain and anorexia are very prevalent subjective symptoms, weight loss and cachexia are more objective signs that are assumed to have prognostic roles in patient survival. Although the prevalence of cachexia varies with the type of malignancy, generally it affects about 50% of cancer patients (2). It has been shown that progressive weight loss in cancer patients leads to poor response to treatment and poor quality of life (2). Therefore, cachexia reduces the long term survival of patients. For all of these reasons, it is vital to understand the mechanisms behind this syndrome.

Two frequently cited definitions of cachexia are: weight loss of more than 5% of pre-illness weight or more than 5% weight loss in the last six months. Many investigations have been conducted to determine the etiology of wasting and cachexia in cancer patients. In general, the results have shown that wasting in cancer cachexia is primarily due to the action of tumor released mediators such as cytokines. These mediators act as proteolytic factors, while some are responsible for lipolysis. Therefore, unlike starvation, which is mainly associated with loss of adipose tissue, progressive and involuntary weight loss in cachexia affects both muscle and adipose tissue (3). The wasting process particularly affects skeletal muscle, and it leads to extreme fatigue and weakness. One of the consequences of muscle fatigue is dyspnea, a common symptom seen in up to 78% of advanced cancer patients (4).

Body composition change in cancer cachexia is an important area of investigation. Previous studies have used indirect methods such as bioelectrical impedance, skinfold thickness, and total body nitrogen, to measure body composition in patients with cancer cachexia (5;6). However, the focus has been mainly on change in lean body mass with less attention to adipose tissue.

Other studies investigated the role of sex and age in the prevalence and prognosis of cancer (7-9). These studies documented that the male gender and older age are predisposing factors for weight loss and poor prognosis. Although one study showed that muscle wasting is greater than that of adipose tissue loss (3), the composition of the loss (ie. muscle type, subcutaneous versus visceral adipose tissue) and the rate of muscle and adipose tissue wasting is not clear. In addition, no study has investigated the rate of change in these tissues in male and female cancer patients.

If wasting of skeletal muscle and adipose tissue are important determinants of performance and survival, then understanding the trajectory of body composition change in cancer is of critical importance and clinically relevant. This information will provide the scientific evidence to support targeted intervention strategies to prevent or diminish cancer cachexia.

B. Purpose

The overall purpose of the current research was to investigate the composition of body weight change in cancer patients over time, during the course of their illness. The relationship between body composition change and nutritional intake was also explored. The study was composed of two parts:

1. A retrospective assessment of body composition change in lung cancer patients aged 40-80 years old by analyzing available CT scans.
2. A prospective assessment of body composition change in cancer patients over the period of 4-6 weeks using DEXA.

Both groups were assessed compared to baseline, and they acted as their own control. Both CT and DEXA scan methods, which were used in this study, are considered the most accurate means of measuring body composition. In both groups indices of body composition (muscle and adipose tissues) were measured.

C. Hypotheses

The research hypotheses for this study were:

1. Patients with advanced cancer experience a higher rate of muscle loss than adipose mass loss.

2. Composition of tissue loss is different between males and females. Male cancer patients experience a higher rate of muscle loss compared to female cancer patients.

3. Muscle loss in cancer cachexia is associated with low protein/energy intake.

D. Objectives

The objectives of the study were:

1. To examine the rate of change of different tissues over time

    -Using CT scan in a retrospective study

    -Using DEXA in a prospective study

2. To investigate the rate of tissue change in different genders

3. To assess the relationship between nutritional intake and body composition change

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Chapter Two

Literature Review

Knowledge in the field of body composition has expanded over the last forty years. It is well established that there are more than 30 components in the human body, which have been classified into 5 major subgroups of atomic, molecular, cellular, tissue systems, and whole body. Among these classes, molecular and tissue systems are the most common models used in the nutritional field (1).

In molecular models, body components are grouped into five distinct compartments including water, lipid, protein, carbohydrate (glycogen), and minerals. However, in the tissue system model, the four major compartments are adipose tissue, skeletal muscle, visceral organ and residual, and skeleton.

Relevant measurement methods for molecular and tissue system models are different. For example, body composition in the molecular model is most appropriately measured by bioimpedance analysis and dual energy X-ray absorptiometery. However, the preferred measurements for tissue system models are computerized axial tomography and magnetic resonance imaging (1). The composition of body components is affected by several factors including sex, age, exercise, diet, and disease.

The next section explains the role of genetic and non-genetic factors on body composition.

A. Factors influencing body composition

Factors, which influence body composition, are classified into modifiable and non-modifiable factors. Although sex and age are non-modifiable, exercise, diet, and disease are considered as modifiable factors.

A.1.Sex

A growing body of knowledge has been accumulated on the gender differences in body composition. The difference between body composition in males and females is mainly due to the effect of sex hormones. In the pre-pubertal stage, body composition is similar for both sexes. As a child experiences puberty, the effect of sex hormones and growth hormone causes changes in body composition. The response is different between girls and boys.

During puberty in males the serum levels of both growth hormone and testosterone are high. High levels of the androgens at puberty are mainly due to the stimulation of gonadothropin secreted from the pituitary gland. Studies have shown that testosterone in turn, enhances the secretion of growth hormone. Both of these hormones are responsible for the growth spurt, symptoms of puberty, and remarkable changes in body composition. Testosterone also plays a major role in protein anabolism. There is evidence that even short-term administration of testosterone can increase whole body protein turnover and whole body protein synthesis (2).

Through puberty in females, increased production of growth hormone is directly related to the level of estradiol concentration. High levels of growth hormone, almost twice that of the pre-pubertal stage increases linear growth in female adolescents (3). However, compared to testosterone, estrogen does not show the same strong potential for protein synthesis (2). It appears that estrogen affects body composition in two ways. While estrogen enhances growth hormone production during puberty, it does not result in a significant effect on whole body protein synthesis in female adolescents. The dichotomous effect of estrogen explains the difference in muscle mass between the sexes.

A.2.Age

Age affects body composition in different ways. Before age 18, body composition is mainly influenced by the effect of hormones. After this age, the secretion of growth hormone and sex hormones decline gradually. It is well known that lean body mass is greatly influenced by free testosterone levels (4). It appears that females lose less muscle mass than males with age (5). However, a detailed investigation showed that the relative

reduction in muscle mass is the same for both genders. In both genders, as age advances, the circulating level of the androgens such as dehydroepiandrosterone (DHEA), and DHEA-sulfate (DHEA-S) declines. Compared to a peak value of DHEA in the 20-30 year old age group, serum DHEA decreases approximately 75% in the 50-60 year old age group. Since skeletal muscle is one of the potential sites for androgen action, the age dependent decline in testosterone level leads to decreased percentage of fat free mass (FFM) but increased percentage of fat mass (FM) (6).

In addition to whole body sarcopenia, with increasing age, the rate of muscle wasting is also different between upper and lower body. Heymsfield et al. (7) showed that independent of gender, in those individuals less than 18 years of age, the skeletal mass percentages in the upper and lower body were not different. On the other hand, in the age group of 45 and older, age and muscle mass are negatively related. As age advances, the muscle mass component decreases. The results of this study also suggest that compared to upper body, the percentage of muscle loss in advancing age is greater in the lower body in both sexes (7).

It is noteworthy that a less active lifestyle and low levels of growth hormone, along with poor dietary intakes may partially be responsible for the wasting process in the elderly.

A.3.Exercise

It is well documented that exercise plays an important role in body composition. Exercise training, in different groups of subjects including children, elderly, or obese individuals, decreases fat mass but increases FFM in general.

Although in the elderly population sarcopenia is a natural process, exercise can slow the rate of loss of lean mass. It seems that older men benefit more from exercise than older women. This could be because men have a higher percentage of lean mass than women (8). However the efficacy of exercise to preserve FFM in the elderly depends on individual compliance.

Children and adolescents are another group who benefit from exercise. DeStefano et al. showed that a 3 month intervention of vigorous aerobic exercise significantly reduced fat mass and increased free fat mass in obese boys. In addition, exercise raises

the level of growth hormone, which in turn stimulates skeletal muscle growth in children (9).

The prognosis of some health problems, including AIDS and cancer, is partially influenced by body composition. Exercise is believed to stimulate protein synthesis and increase muscle mass. A combination of aerobic and resistance training improves body composition of cachexic HIV patients (10).

In cancer patients, the anabolic effect of exercise can reduce muscle wasting and cachexia. It has been shown that exercise not only delays the onset of anorexia and cachexia but also retards tumor growth (11). Al-Majid et al. showed that resistance exercise training through electrical stimulation of the extensor digitorum longus muscle in rats slowed the rate of muscle wasting compared to the control group. However, the control group did not show an increase in muscle content with the exercise. This study suggested that the amount and intensity of the exercise program, which is able to retard the process of muscle wasting in cancer patients, might not be sufficient to induce muscular hypertrophy in healthy subjects (12). It can be concluded that muscle wasting, a poor prognostic factor for cancer survival, may be reduced by exercise intervention.

A.4.Nutritional intake

The effect of diet on body composition starts from the beginning of life even when the fetus is growing in the maternal uterus. Dietary habits and health status of the mother are factors that guide the composition of the fetus.

After infancy, the effect of diet composition on body composition seems to follow a particular pattern throughout the lifespan. Body composition is modified by the quality and the quantity of food. Many studies have been conducted to explain the role of diet in body composition. The majority has focused on the effect of dietary fat. The results of these studies showed that chronic excess fat intake, even a few grams more than the requirement, is sufficient to cause obesity. As such, obesity is a consequence of energy imbalance (energy intake >energy expenditure). Increased fat mass as a result of high fat intake can be explained as following:

1- Unlike protein and carbohydrate, fat oxidation is not as tightly regulated by fat intake. Human studies showed that carbohydrate and protein oxidation are enhanced in response to carbohydrate and protein intake (13-15).
2- Energy substrates other than fat, such as carbohydrates, have an inhibiting effect on fat oxidization (16).
3- High fat intake promotes increased food consumption due to its high energy density (17).
4- Delayed satiety with fat compared to protein and carbohydrate consumption leads to longer eating duration and therefore higher energy intake (18).
5- Meal induced thermogenesis is lower with fat intake compared to protein or carbohydrate intake (19).

Despite the known propensity of malnutrition on cancer survival, nutritional care is not routinely implemented in this population. Patients with advanced cancer often have a reduced food intake. Although for years, low nutrient intake had been blamed for the malnutrition in cancer patients, increased energy and protein intake by enteral or parenteral means failed to show a significant impact on reducing patients' progressive wasting (20-22). However, investigations of a number of specific nutrients in experimental and human models reported relative success in improving weight in people with cancer. Eicosapentaenoic acid (EPA) has been a focus of interest for a number of years. Introducing EPA to mice bearing cachexia-inducing colon adenocarcinoma showed beneficial effects on reducing the rate of weight loss and tumor growth in this animal model (23). Barber et al. administered fish oil containing EPA to pancreatic cancer patients experiencing weight loss for three weeks. They reported that after this period the patient's weight improved. However the increment in lean body mass was not significant (24).

Megestrol acetate (MA) has been established as an appetite stimulator and weight loss inhibitor for the anorexia-cachexia syndrome. Although this agent enhances appetite and food intake, the weight gain is predominantly body fat (25). Despite inducing weight gain, it appears that MA has an antianabolic effect on muscle. In a study that investigated the effect of MA, testosterone, and resistance exercise in elderly men, Lambert et al. concluded that MA may decrease muscle size even in combination with testosterone (26).

A.5.Diseases (Burns, Heart disease, COPD, Anorexia nervosa, Cancer)

Body composition changes occur under particular circumstances such as metabolic stress (burn, injury, surgery) or the presence of diseases such as diabetes, HIV, and cancer. Following a metabolic stress two consecutive metabolic responses occur: the "Ebb phase" and the "Flow phase". Immediately after injury, the Ebb phase is characterized by low cardiac output and decreased metabolic rate. At this point, aggressive fluid and electrolyte replacement is required for survival. As soon as the patient is stabilized, the flow phase begins. At this stage, the patient experiences hypercatabolism. The hypercatabolic state is represented by enhanced skeletal muscle protein breakdown, which in turn increases the amino acid release into circulation. Following an injury, urinary nitrogen excretion increases. The accelerated nitrogen excretion is due to increased mobilization of amino acids from skeletal muscle. It has been shown that during the acute phase of injury, the extent of amino acid released from skeletal muscle is greatly dependent on the general metabolic response of the body to the injury itself and the extent of total body surface area of the injury (27).

During the flow phase, high serum levels of amino acids are used for two purposes. The majority of these circulatory amino acids are used to synthesize acute phase proteins that are necessary for the inflammatory response and tissue reconstruction. The second purpose is that amino acids are taken up by the liver to produce glucose via the gluconeogenesis pathway. Therefore, the protein turnover responds to injury in a manner that redistributes body protein from skeletal muscle to satisfy other more urgent protein needs. The outcome is wasting of muscle mass through the catabolic stage of injury.

The metabolic response to injury or burns is exaggerated in children. Neonates and children not only have less protein stores compared to adults but they experience high energy requirements for growth. Therefore seriously injured younger children are at higher risk of the deleterious effects of hypercatabolism which continues through to the rehabilitation stage of management (28).

In the following sections the effect of various clinical situations on body composition is explained briefly.

A.5.1.Burns

In severe burns a chain of metabolic and dynamic responses occur which include: hyperdynamic circulation, fever, immune deficiency, peripheral insulin resistance, impaired hepatic protein production, enhanced muscular protein catabolism, and elevated energy expenditure. Systemic energy expenditure is well correlated with the degree of muscle protein catabolism, impaired wound healing and immune deficiency (29).

The results of a study of pediatric burn patients, Hart et al. showed that age, sex, and weight are significant markers in predicting metabolic rate after injury (30). The degree of burn (total burn size, percent of full thickness burn, wound area) and severity of inhalation injury are directly related to the metabolic rate. They also found that protein catabolism is synonymous with the acute phase of injury. It appears that protein catabolism supplies substrate for the liver (to enhance the protective acute phase protein response) and to skin (to stimulate cutaneous protein synthesis).

Investigations of the effect of nutritional support in burns showed that early and aggressive enteral nutritional intervention attenuates auto-catabolism after burns. Relative to systemic energy expenditure, broad spectrum nutritional energy delivery makes no difference in the rate of muscle protein catabolism. Increased feeding has a strong linear correlation with deposition of fat mass. Therefore, it is concluded that increasing caloric balance, in pediatric patients, maintains weight only via increasing fat deposition in the face of continued lean muscle mass loss. In spite of high protein and energy nutritional support, erosion of lean body mass continues for at least 9 months after injury in patients with severe burns (40% TBSA) (30). This study did not show any association between increased nutritional supplementation and mortality. Other studies also have shown that caloric delivery more than 1.2 times of resting energy expenditure is associated with increased fat mass without change in fat free mass (31).

A.5.2.Cardiac Cachexia

In severe chronic heart failure (CHF), marked weight loss, muscle weakness, and fatigue are important manifestations (32).In 1994, Freeman et al. defined cardiac cachexia as a loss of $\geq$ 10% lean tissue (33). However, Anker et al. (34) proposed other criteria for cardiac cachexia which are as follows:

1-Duration of CHF for at least 6 months.

2-No sign of other primary causes of cachexia such as cancer, thyroid disease or severe liver disease.

3-Weight loss of > 7.5% of the previous normal weight.

The pathophysiology of cardiac cachexia remains uncertain. Neuroendocrine markers and immunological abnormalities are initial indicators of this condition. Although there is evidence that loss of muscle tissue contributes to the impaired blood flow and reduced oxidation capacity in CHF patients (35), other etiologic factors such as anorexia and reduced food intake, a sedentary lifestyle, increased serum TNF-α (36) and increased serum cathecolamines (37) seem to be important etiologic factors in developing cachexia and muscle wasting in heart failure patients.

A.5.3.Chronic Obstructive Pulmonary Disease (COPD)

Weight loss in COPD patients is mainly due to hypermetabolism and low energy intake. Hypermetabolism arises from the increased oxygen requirement of respiratory muscles, which is related to high airway resistance and increased minute ventilation. In this condition, in the presence of hypoxemia, ATP production is reduced. Therefore energy production relies on anaerobic sources (38). Reduced caloric intake in the absence of malabsorption is a common cause of nutritional depletion in COPD patients. These patients become exhausted when they eat large amounts of food. In addition, a higher caloric intake is associated with increased CO2 production, which in turn augments ventilation to levels intolerable for patients with dyspnea (39).

Elevated concentration of pro-inflammatory cytokines (TNF-α and IL-1β) could be another explanation for weight loss in COPD patients. These cytokines, as will be explained later, are associated with accelerated metabolism and protein turnover (40). Weight loss in COPD includes muscle wasting as well as fat depletion. Bioelectrical impedance assessment (41) and MRI studies (42) have shown that compared to matched control subjects, fat free mass is reduced in patients with COPD.

A.5.4.Anorexia Nervosa (AN)

Starvation and cachexia are known as the two major paradigms of malnutrition. Although cachexia, as in neoplastic and cardiac conditions, is characterized by inflammatory responses, the main etiology of starvation in anorexia nervosa (AN) is pure caloric deficiency. Therefore re-feeding the patient will reverse the process of malnutrition in starvation but not in cachexia. In starvation, body metabolism adapts in a manner that conserves fat free mass and enhances fat metabolism to meet basal energy requirements. To conserve additional energy, total energy expenditure, resting energy expenditure and anabolism decline. Although starvation is known as an outcome of low energy intake, it has two different etiologies, non-intentional and intentional. Intestinal disease with malabsorption is an example of the former. In contrast, anorexia nervosa and dieting are intentional types of starvation.

Anorexia Nervosa is a common, chronic malnutrition seen in western countries. This condition affects 0.5% of adolescent girls aged 15-19 years with mortality as high as 20% if left untreated (43).

In 1996, Prosb et al. examined the body composition of females with AN using skinfold thickness and underwater weighing. The result of this study showed that the percentage of body fat is mainly related to the subtype of the eating disorder. The fat mass percentage was higher in patients with A.N who binged and purged than those who were the restrictive type of AN (44).

Later in 2002, dual-energy x-ray absorptiometry (DEXA) was used to assess body composition of adolescent AN patients (45). This study reported that patients with AN had 12.5% less body fat mass than a control group. Comparison between the trunk and legs revealed that these patients lost more abdominal fat than peripheral limb fat. In addition to fat wasting, fat free mass was also depleted in this group of patients with AN. (mean=34.5 kg in AN vs mean=41.2 kg in the control group). However, as a feature of starvation, re-feeding anorectic patients restores fat mass, fat free mass, and extracellular water.

In 1997, Orphanidou et al. investigated body composition and fat distribution changes after short term weight gain in 26 females with anorexia nervosa. The result showed that a mean weight gain of 6.7 kg included an increase in body fat, as well as lean

body mass and bone mineral mass. However, fat remained the main component of weight gain. This study also showed that there was no preferential fat deposition in the three main central regions of the body (subscapular, waist, and thigh) (46).

A.5.5.Cancer Cachexia

Cachexia, a well-known complication of many life-threatening diseases, is seen in most patients with advanced cancer. Although the definition of cachexia varies, cancer cachexia usually entails weight loss of more than 5% of body weight during the previous 2-6 months or a loss of more than 5% of the pre-morbid weight. Cachexia is a syndrome that is mainly characterized by progressive weight loss and hyper-catabolism, which results in loss of body tissue such as skeletal muscle and adipose tissue. Anorexia, weakness, poor performance, and impaired immune function are other symptoms that accompany wasting in advanced malignancy. Cachexia is commonly considered a major contributing cause of death in cancer patients (47).

Important information about the role of tumor type, tumor stage and cachexia, the prognostic effect of weight loss, and the treatment response in terminal cancer patients came from a study conducted by Dewys et al. (48) In this literature, the reported incidence for cachexia is highly variable according to the type of tumor, tumor stage, and tumor mass. Among 3047 cancer patients who participated in different ECOG (Eastern Cooperative Oncology Group) studies, patients with breast cancer, acute non-lymphocytic leukemia, and non-Hodgkin's lymphomas had the lowest frequency of weight loss (30-40%). Patients with colon, prostate and lung cancer had a moderate frequency of cachexia (48-61%), and 83-87% of patients with pancreatic and gastric malignancies had the highest frequency of cachexia. The data derived from the ECOG study suggested that the incidence of weight loss is higher in patients with solid tumors than those with hematological malignancies. The ECOG study also examined the relationship between cachexia and tumor stage. In this study, advanced cancer was considered tumor metastasis to the liver, lung, or bone and tumor extent was scored by the number of organs involved (bone, liver, and lung). The results showed that in sarcoma and colon cancer there was a 10% increase in weight loss frequency when more

than two organs were involved, but only breast cancer patients with more than two organs involved were statistically more likely to have significant weight loss.

B. Prognostic significance of weight loss

It appears that weight loss is associated with poor prognosis at every stage of the disease trajectory. In the study by Dewys et al. (48) weight loss was also seen to be of prognostic significance. A weight loss of > 5% of body weight at the time of diagnosis compared to no weight loss was associated with a significantly poorer median survival, chemotherapy response, and performance status (0=fully active, 1=ambulatory, 2=in bed<50%of time, 3=in bed>50% of time, 4=completely bedridden). Weight loss was associated with decreased median survival within performance status categories as well as within categories of anatomic involvement. These observations emphasize the prognostic effect of weight loss, even in patients with a favourable performance status or a limited anatomic involvement with the tumor.

The prognostic significance of fat versus lean tissue loss has not been explicitly established, although it is a widely held belief that loss of metabolically active lean tissue contributes to increased mortality, accelerated disease progression, and impairment of strength and functional status (48). This concept underlies the targeting of a variety of protein anabolic agents in patients with wasting syndromes.

C. Experimental and clinical research

C.1. ANIMAL STUDIES

C.1.1. Skeletal muscle atrophy

The evidence for muscle atrophy in cancer cachexia comes from experimental and clinical studies. Wasting of skeletal muscle and possibility of increased proteolysis has been studied in animal models. In one study conducted by Llovera et al., rats were injected with Yoshida AH-130 hepatoma and then were evaluated after 7 days. These investigators reported a 30% decline in muscle mass in gastrocnemius and extensor

digitorum longus (EDL) muscle in tumor bearing rats compared to the control group. They suggested that muscle wasting is due to the increased proteolysis mediated by ubiquitin-dependent proteases (49).

Significant muscle loss is apparent early, as an individual starts to lose weight and it is more or less continuous thereafter. Animal studies have documented that abnormalities in protein synthesis and degradation are evident at the earliest stages of muscle wasting (50).

In addition to increased proteolysis as a major factor of muscle wasting, experimental studies have also shown that the composition of contractile protein isoforms (myosin) is also changed in cachexia. Some muscles have a unique composition of two main myosin isoforms, fast twitch and slow twitch. For example the soleus muscle is composed of almost 95% slow oxidative fibres, while the extensor digitorum longus (EDL) has almost 95% fast glycolytic fibres. When the murine C-26 adenocarcinoma cells were introduced to mice, the soleus muscle showed shifts in myosin isoform content. This change was characterized by increased type IIb MHC (myosin heavy chain) fast twitch fibers, from an undetectable expression level in the control group composed to 19% in tumor bearing mice. The results of this study showed that muscle wasting, which accompanies cancer is associated with a shift in myosin isoform content of some muscles, especially the soleus muscle (51).

Another factor that contributes to tissue atrophy in cachexia is immobility. It is well known that muscle disuse and a sedentary lifestyle, induces a considerable degree of muscle atrophy in normal healthy animals (52), as does bed rest in humans (53).

C.1.2.Adipose tissue loss

Wasting of adipose tissue is another component of weight loss in cachectic syndromes. Alteration in lipid metabolism is the main reason for loss of body fat. In animal models, elevated levels of circulating triglycerides and non-essential fatty acids have been shown to be associated with decreased activity of the enzyme lipoprotein lipase. Most changes in lipid profiles are related to the reduced activity of this enzyme. Several cytokines such as TNF-α, IL-6, and INF-γ are thought to be responsible for fat mobilization and fat oxidation in cancer through inhibition of the lipoprotein lipase.

Strassman et al. (54) have reviewed the involvement of cytokines in cancer cachexia and they describe the contribution of IL-6 and other cytokines to the wasting observed in C-26 adenocarcinoma bearing mice. The neutralization of IL-6 by antibodies, or IL-6 receptor antagonism by suramin (an anti-cancer agent), significantly reduced the severity of key parameters of cachexia.

C.2.HUMAN STUDIES

A number of studies conducted in different cancer patient populations have identified alterations in the balance between anabolic and catabolic processes in adipose tissue (lipogenesis and lipolysis) and skeletal muscle tissue (protein synthesis and proteolysis).

C.2.1. Skeletal muscle atrophy

Muscle mass depends on the balance between the rate of protein degradation and protein synthesis. In general, an increased rate of whole body amino acid turnover is considered the key mechanism involved in muscle wasting. Evaluation of whole body protein kinetics is composed of two compartments, the skeletal muscle and non-skeletal muscle protein. To distinguish whole body protein kinetics from skeletal muscle protein kinetics Deworzak et al. (55) conducted a study in which they investigated whole body protein and skeletal muscle protein synthesis and breakdown in cachectic patients with advanced gastric carcinoma and in healthy volunteers. The postabsorptive state of constant infusion of L-[$^2H_5$] phenylalanine and L-[$^2H_4$] tyrosine was used to determine the isotopic concentration in plasma during the plateau phase using gas chromatography. The results showed that there was no significant difference in the rate of whole body protein synthesis and breakdown between the two groups. Also no meaningful difference was seen in skeletal muscle protein breakdown between groups. However the skeletal muscle protein synthesis rate in the patient group was significantly lower than the control group. Decreased protein synthesis in cachexia could result from reduced insulin sensitivity of skeletal muscle, decreased insulin concentration in plasma, or reduced amino acid supply required for protein synthesis. In contrast to reduced skeletal muscle

protein synthesis, hepatic protein production is enhanced in cachexia (56), which may limit the availability of amino acids for skeletal muscle protein production.

In 1998 McMillan et al. studied body cell mass depletion in 12 cancer patients over 12 weeks (57). Their purpose was to assess the relationship between an inflammatory response and muscle wasting in cancer cachexia. In this study body cell mass (BCM) was estimated using the following formula:

BCM (kg)= total body potassium (mmol)×0.00833.

They also measured C-reactive protein as an index of an inflammatory response. Baseline characteristics of patients revealed that the median weight loss was 11.7%. Total body potassium values were below predicted and C-reactive protein values were elevated. There was a significant inverse relationship between C-RP concentration and total body potassium over a 12 week follow up period. In those patients who lost body potassium (TBK), approximately 7% loss of TBK (equivalent to approximately 1.5 kg of BCM) was correlated to C-RP concentration of 40 mg/l.

Physical inactivity (a common complication seen in cancer patients) is another cause for muscle wasting. In 1995, Ferrando et al. (53), investigated the effect of bed rest on fat free mass. Since MRI is able to detect small changes in tissue volume, this method was used to determine regional muscle volume change. Five young, healthy, male volunteers were subjected to seven days complete bed rest. MRI images were taken from calves and thighs of each participant, before and after the bed rest period. MRI analyses demonstrated that 7 days of bed rest induced a significant decrease in thigh muscle volume (3%).

C.2.2. Adipose tissue loss

It has been shown that in cancer patients who experience weight loss, fasting plasma glycerol level is higher than in weight stable cancer patients. This provides evidence for an increase in lipolysis (58). Shaw and Wolfe compared glycerol and free fatty acid (FFA) kinetics in normal volunteers, non-weight-losing gastrointestinal cancer patients, weight-losing gastrointestinal cancer patients, and in severely septic patients by using constant infusions of d-glycerol and 1-^{13}C palmitic acid. The results showed that in terms of lipid kinetics, non-weight-losing cancer patients were not significantly different

from healthy volunteers. However weight-losing cancer patients and septic patients had elevated rates of lipolysis. In contrast to what was seen in non-weight losing cancer patients and healthy volunteers, glucose infusion in weight-losing cancer patients and in septic patients did not result in a significant inhibition of lipolysis.

The mechanisms of altered fat metabolism in cancer cachexia are not yet confirmed. In 1993, Thompson et al. (59) conducted a study to investigate the expression of gene coding for those enzymes that seemed to be involved in uptake, synthesis, and metabolism of lipid in cancer patients. Lipoprotein lipase enzyme activity and levels of mRNA were measured in both cancer and control groups. These investigators did not detect any difference in serum TNF-α, total lipoprotein lipase enzyme activity (LLA) and relative level of mRNA for the LLA between cancer and control groups. However, they found two-fold increase in relative level of mRNA for hormone-sensitive lipase (HSL) in adipose tissue (subcutaneous) and two-fold increases in serum triacylglycerol and serum free fatty acid levels of the cancer group compared to the control group. They concluded that increased expression of HSL is one of the main factors that is responsible for depletion of lipid from adipose tissues in cancer patients.

In 1990, Groundwater and coworkers (60) proposed that some tumors may secrete an unusual Lipid Mobilizing Factor (LMF). LMFs act directly on adipose tissue by releasing free fatty acids and glycerol, the same way that lipolytic enzymes act. The work of this group showed the activity of a LMF found in the urine of cachectic patients correlates with the extent of weight loss.

Collectively, the results presented above suggest that cancer progression is associated with muscle and fat mobilization, driven by an imbalance between anabolic and catabolic processes. However, many features of cancer-associated tissue loss in patients remain unclear. The relative proportions of fat and muscle loss and their time course are not known. The data are often acquired at a single time point, and it is unknown whether tissue depletion may be continuous, episodic or both. It is not known whether specific fat depots or skeletal muscles are more or less affected. The functional implications of wasting of specific muscles could be important. Loss of muscles in the limbs can affect activity and work, while loss of respiratory muscle is associated with dyspnea.

D. History of body composition measurement in cancer cachexia

In many studies involving the measurement of body composition, indirect methods such as skinfolds (61), total body nitrogen (62), total body potassium (57), and Bioimpedence analysis(BIA) (63) have been used. In recent years these indirect methods have been replaced by more accurate techniques such as dual-energy x-ray absorptiometry (DEXA) (64), magnetic resonance imaging (MRI) (7), and computed tomography (CT) (65). In the last few decades a significant amount of research has been done to investigate the etiology and mechanisms of wasting in cancer patients. However the nature and composition of weight loss, and the rate of fat and muscle mass change, is still debated. The literature reveals that indirect methods are the most common methods used to measure fat free mass (FFM), and fat mass (FM) in cancer patients.

Important information about body composition in cancer patients came from the work of Cohn et al. (66). In their study, a compartment analysis technique was used to assess the composition of FFM and FM. This technique was a combination of the prompt gamma neutron activation technique to measure total body nitrogen, a whole body counter technique to measure total body potassium, and a tritium label to measure total body water. Data showed that although cancer patients lost both fat and muscle, these patients tended to preserve body fat mass better than FFM. Therefore it was concluded that compared to healthy subjects, skeletal muscle is the predominant wasted tissue in cancer cachexia.

Later, in 1990, Fearon et al. (62) investigated the body composition of lung cancer patients using biochemical methods. Total body nitrogen, chlorine, calcium, and phosphorus were measured by neutron activation analysis, and a whole body counter measured total body potassium. Unlike Cohn's study, this study demonstrated that in cachectic lung cancer patients wasting of body fat mass was greater than lean body mass (80% vs 75%).

The difference between these two results may originate from the fact that in the former study, a heterogeneous group of cancer patients was assessed, while in the later study, a specific cancer type was investigated. In 1997, Fredix et al. (67) conducted a study in which the body composition of non-small cell lung cancer patients was assessed

by BIA and skinfold thickness methods before and after the resection of their tumor. In both groups the tumor was removed at the time of study. The two groups were different based on whether or not they experienced tumor recurrence. The body composition changes were measured over a period of one year. The result re-emphasized the wasting nature of cancer. Patients with tumor recurrence lost weight, especially from FFM. In contrast, patients with no sign of tumor recurrence gained weight predominantly in fat mass rather than FFM.

Imaging techniques are the most precise methods of measuring FFM and FM. Among these techniques, DEXA is well known as the safest method with regard to the radiation dose that the patient is exposed to. To investigate the relationship between weight loss and the existence of low body cell mass, 20 lung cancer patients were studied by Simon et al. (68). These patients were divided into two groups of 10 subjects. One group experienced a weight loss of greater than 10% of usual body weight and the other group consisted of subjects with a weight loss of less than 10% of usual body weight. Bone mass, FFM and FM were measured in all subjects using DEXA. Although weight, fat mass, and body cell mass were lower in the group with weight loss of more than 10%, the difference between the two groups was not statistically significant.

When studying body composition in cancer, one should also consider the effect of treatment, sex, and age. It has been shown that sex and treatment significantly modify the nature of body composition changes and survival in cancer patients (69). It has also been reported that males are more susceptible to loss of weight, muscle, and fat than females. Therefore, men manifest shorter survival rates compared to women (69;70).

E. Nutritional support in cancer cachexia

In patients suffering from cancer, malnutrition is a distressing complication, which is associated with refraction to treatment, poor quality of life, and increased morbidity and mortality. Weight loss, tissue wasting, suppressed appetite, nausea and vomiting, and food aversion characterize the anorexia-cachexia syndrome. Thoresen et al. (71)suggested that etiological factors of cancer malnutrition should be divided into primary and secondary causes. Primary factors mainly refer to malnutrition due to hypermetabolism, and the function of various cytokines. Several cytokines, mainly

interleukin (IL)-1, IL-2, IL-6 and tumour necrosis factor alpha (TNF α), are known to be involved in the pathogenesis of the anorexia-cachexia syndrome (72). Additionally, neurotransmitters including serotonin (5-HT), which are produced or released by peripheral blood cells, play a significant role in developing nausea and vomiting, which in turn exacerbates anorexia (73).

On the other hand, secondary etiological factors for malnutrition include mechanical obstruction, malabsorption and psychological factors. Some malignancies result in mechanical obstruction of the GI tract. These obstructions interfere with swallowing of food (in head, neck, and mouth cancer) or with food passage through the GI (as seen in esophagus, gastric, pancreatic, and colon cancer). In these cases the surgical resection or bypass is applied to increase food intake. Although nausea and vomiting (N/V) are mainly mediated by the action of neurotransmitters on the central nervous system, in some patients N/V is an outcome of gastrointestinal obstruction or is a result of autonomic dysfunction, which leads to gastric stasis and eventually delays gastric emptying. Inflammation in the gastrointestinal tract is an unfortunate side effect of cancer treatment. Inflammation seen in the small intestine after radiotherapy causes impaired intestinal absorption of macro and micronutrients.

Although anorexia, a common complication of advanced cancer, is itself a consequence of changed metabolism and cancer treatment, it can also be considered secondary to wasting and immobility. Therefore, whether anorexia is primary or secondary to cachexia remains unclear.

Some investigations have shown that nutritional intervention may delay the progression of malnutrition and fat and muscle wasting in these patients. Assuming that prevention of weight loss leads to improvement of survival and quality of life, early detection of weight loss is an important goal. Nutritional status of cancer patients should be assessed and monitored to determine the patient's response to the disease, treatment, or nutritional intervention. Routinely a combination of different methods is used to evaluate nutritional status.

Anthropometric tests are one of the objective and commonly used methods of assessment of body composition. Body weight is a simple index of body mass. For years, it was assumed that percentage of weight loss is an indication for malnutrition. In fact,

the definition of cachexia has been established based on the percentage of weight loss for a given period of time. However, increases in extracellular fluid in the form of edema and ascitis and/or increases in tumor mass limits its validity to assess nutritional status.

Laboratory tests such as plasma protein analysis, urinary metabolite excretion, and body composition methods are other objective parameters of assessing nutritional status. In regards to time and cost, in some clinical settings these strategies may not be practical. In these situations, other methods must be used.

To overcome some of these disadvantages, Detsky et al. (74), developed the subjective global assessment (SGA) questionnaire, which enabled researchers to assess nutritional status based on patient history and physical examination. Later, based on the original SGA questionnaire, Ottery et al. (75) designed a new questionnaire for cancer patients, which was called Patient Generated-Subjective Global Assessment (PG-SGA). PG-SGA has been accepted as a valid tool to investigate nutritional status in the cancer population (76).

There are few reliable ways to assess energy intake. For decades, dietary records have been used to evaluate energy and nutrient intake (77). Two types of dietary records are used in research including a weighed or un-weighed dietary record. A weighed dietary record is the optimal way of assessing energy and nutrient. In this method, the actual weight of all the food and drinks are recorded at the time of consumption (78). Although, this method is the most accurate way of measuring energy intake, it is not necessarily a feasible and practical approach in some health conditions including cancer. Therefore, in many research and clinical settings, weighed dietary records are substituted by unweighed dietary records. In this method, patients are asked to record food intake by estimating the portion size according to household measures. Depending on the research protocol, patients are instructed to complete a 1, 3, 4, or 7 day food record (79-81). The final step in assessment of energy and nutrient intake is to analyze the dietary records by using a nutrient analysis software program. These computerized tools provide daily intake values of macronutrients, micronutrients, and energy.

F. Body composition measurement methodology

To evaluate tissue wasting in cancer, there are several important methodological considerations. First, tissue wasting occurs at both the whole body level and localized to certain parts of the body. Second, sensitive measures are required to follow outcomes of therapeutic interventions directed at attenuation of fat and muscle wasting. Third, it is widely believed that skeletal muscle depletion is the most important factor in wasting syndromes, and most treatments are directed towards maintenance (or ideally synthesis) of lean muscle mass (82;83). For this reason, measures of muscle/lean mass are a particular focus of the research literature on wasting syndromes (84).

Over the past few decades, several methods have been developed to qualify and quantify total and regional body compartments with the most emphasis on fat free mass. Body composition measurements could be categorized in three major groups including:

1) Anthropometric tests such as body weight, skinfold thickness, and mid-arm circumference.

2) Bioimpedance/resistance and electroconductivity.

3) Imaging techniques: CT, MRI, Ultrasound scanning and DEXA.

F.1. Anthropometric tests

-**Body weight** is an index of body mass. It is generally accepted that because of the change in fluid status and the variable utilization of fat and lean mass for covering metabolic demands during disease, body weight change does not precisely reflect the composition change of a patient's body wasting.

-**Skinfold thickness** is used to estimate body fat. Triceps skinfold, biceps skinfold, subscapular and suprailiac skinfolds are standard sampling sites. Observer expertise, instrument error, subject's age and fitness and total body water are sources of error in this technique.

-**Mid-arm circumference** in combination with the value of triceps skinfold is used to estimate the mid-arm muscle circumference. Arm muscle area ($AMAcm^2$) is calculated from triceps skinfold thickness (TSF, cm), and mid-arm circumference (MAC, cm). In assessing the accuracy of the current equation by comparison to AMA measured

by computerized axial tomography, error in each of the four approximations made was found to result in a 20 to 25% overestimate of AMA. After correcting for these errors, this technique can be used as a bedside measurement of BCM (85). Besides these errors, one important limitation of this approach is that it is limited to changes in muscle area in the upper arm only and thus may or may not reflect changes in the trunk or lower limb.

F.2. Bioimpedance/ electroconductivity analysis:

This method measures resistance of the body to the flow of an alternating electrical current. Electroconductivity works based on the principle that lean tissue conducts an electrical current better than adipose tissue. Although BIA is a rapid and safe bedside technique it is mostly used to measure resistance at the extremities and therefore is less accurate in assessing body composition of patients whose weight loss is predominantly from their viscera or abdomen. However, by using accurate equations, compared to DEXA, BIA provides a reasonable estimate of percentage of body compartments (86). This approach has the limitation that it cannot discriminate between local and regional body composition.

F.3. Research techniques (imaging technology)

Skeletal muscle is a large component of body composition in humans and it plays a significant role in physical activity and many biochemical processes. There has been an increasing interest in studying muscle distribution and regional muscle mass over the past decade. At present, the most accurate in vivo methods of measuring segmental body composition are DEXA, CT and MRI.

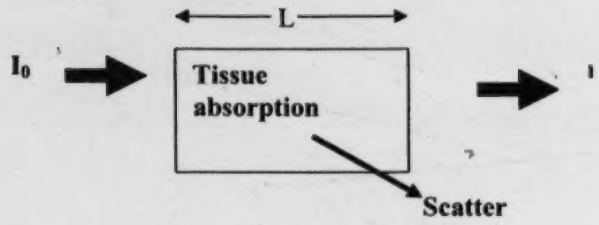
-DEXA: is becoming one of the most readily available ways of measuring body composition. The advantage of DEXA is its lower cost and less radiation exposure compared to CT and MRI.

Physical concepts of DEXA: DEXA scanning is currently used to measure bone mineral density in clinical settings and to measure whole or regional body composition in research.

DEXA works based on the absorption of photons as they pass through material or tissue. Therefore, photon absorptiometry requires a photon source and detector. In the earliest DEXA instruments, monoenergetic radionuclide sources such as ^{125}I and ^{241}Am were used to produce photons (87). These single energy sources were mainly used to evaluate the bone mineral. Later, dual energy sources such as ^{109}Cd replaced the monogenic radionuclide source in order to measure soft tissue (88). An important advance in photon absorptiometry was replacement of radionuclide source with X-ray sources. X-ray is an electromagnetic radiation of short wavelength, which is produced when high-speed electrons strike a heavy metal. The x-rays consist of a broad spectrum of photon energies ranging from 15 keV to 80 keV. If tungsten is used as a decelerator, two small peaks will be seen in the energy spectrum at 55keV and 70 keV.

An approach to create the required two main energy peaks is to use"k-edge" filters. These filters are basically made of rare earth materials such as cerium (Ce) and samarium(Sm). In each atom, the electrons are arranged as concentric shells. The innermost shell (k-shell electron) can be ejected if photon energies are equal or slightly higher than the energy level of k-shell electron. Therefore, these photons attenuate as they interact with k-shell electrons. This process is called "absorption edge". Depending on what absorption edge is required, certain elements can be used. For example, the k-edge characteristics of cerium are 38-40 keV and 70 keV, while these peaks for samarium are 46.85 keV and 80 keV (89).

As a beam of photons pass through tissues, the attenuation phenomen occurs,which means a fraction of the photon stream is either absorbed or scattered. The result is the reduction of beam intensity. The diminshed beam density is then recorded by the detector.



As the proton beam passes a homogenous absorber, the beam intensity is reduced. The reduced fraction of the intensity is a function of linear attenuation coefficient (μ) and path length (L)(89).

$$-d(I/I_0)= \mu \times dL$$

According to the above equation, intensity of the transmitted beam is proportional to initial photon intensity, path length, and linear attenuation coefficient. On the other hand, the linear attenuation coefficient is density (ρ) dependent. Thus, in heterogeneous tissue with different densities, mass attenuation coefficient (μ_m) or μ / ρ represents the linear attenuation accurately. From the above information, it can be concluded that attenuation is negatively related to the photon energy intensity but correlates positively with the mass attenuation coefficient (89).

When a tissue is exposed to two different energies from a photon beam, the ratio of attenuation at lower energy to the attenuation of higher energy is shown as R (ratio value) (90). For a homogenous absorber, R is a known and constant value. However for a heterogenous absorber, R is a function of mass attenuation coefficient and mass fraction of each component. In the DEXA system, R value is used to identify unknown component.

In the DEXA approach, the human body is first assumed to be composed of two compartments: bone mineral and soft tissue (fat + lean soft tissue). This segmentation is based on the distinct range of x-ray attenuation. Further, the system distinguishes the soft tissue to fat and lean tissue, according to the R value for the two respective components.

In summary, in this method photons are radiated from an x-ray source and then scanned over the patient at two different energy levels. The differential absorption of

photons is measured. DEXA first separates the whole body into soft tissue and bone mineral. Further, soft tissue is separated into FFM and fat by use of the ratio of x-ray attenuation (91).

To validate the accuracy of DEXA in measuring regional skeletal muscle, the DEXA-SM model was compared with corresponding CT-measured regional appendicular skeletal muscle (fat free) (92). For this purpose 27 male subjects (twenty were healthy and seven had AIDs) completed DEXA and CT studies. Identical landmarks in calves, thighs, and forearms were selected for both measurements. Trunk SM was not evaluated because DEXA is unable to differentiate skeletal muscle in the trunk from other lean tissues such as organ tissues. The results showed that there is a strong correlation for fat free skeletal muscle between DEXA and CT methods ($P<0.001$).

Thus, this study showed that DEXA measured regional skeletal muscle mass similar in magnitude to the skeletal muscle measured by a CT scan in men. More studies are required to assess the model's applicability to measure skeletal muscle in other regions or with other subject groups such as women and the elderly.

-Computerized tomography/Magnetic resonance imaging: The most important advantage of MRI and CT is direct visualization of images from the skeletal muscle cross- sectional area. These two methods are the most accurate and promising reference methods that can be used for measuring whole body and regional skeletal muscle mass. In 1998, Mitsiopoulos et al. (93) proposed a practical use of MRI and CT scanning, which can be used in clinical settings to quantify total and regional skeletal muscle volume as well as interstitial adipose tissue and subcutaneous adipose tissue. In their study, they calculated cross-sectional skeletal muscle and fat tissue of leg and arm sites and then compared the values with corresponding cadaver estimates. A combination of methods was used to determine muscle and fat tissue area. These methods included edge detection filters and watershed techniques. Once the regions representing the various tissues were identified, a mouse pointer was used to identify each tissue by using color codes. Each image was reviewed by using an interactive slice-editor program that allows for verification and correction of the segmentation result where necessary. To calculate tissue area (cm^2), the respective tissue region in each slice was computed automatically by summing the given tissue's pixels and multiplying by the pixel surface area. Tissue

volume (cm^3) for each slice was calculated by multiplying the tissue area by slice thickness. The findings of this study showed that:

1-Both MRI and CT estimates of FFM and FM are highly correlated with corresponding cadaver values. ($P<0.001$) and

2-It also confirmed that CT and MRI are precise methods for estimating appendicular fat free skeletal muscle, subcutaneous fat, and interstitial adipose tissue in humans.

3-The reproducibility for these two methods in estimating fat free skeletal muscle and subcutaneous fat is 2%. That makes them the best methods of detecting small changes in body compartments in both cross sectional and longitudinal studies.

In summary, understanding the nature of wasting in cancer cachexia requires more research to investigate body composition changes more precisely. This aim can be achieved by using variety of methods especially imaging techniques.

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Chapter Three

Evaluation of regional adipose tissue and skeletal muscle loss during progression of non-small cell lung cancer using CT image analysis

Introduction

Although the definition of cachexia varies, cachexia entails weight loss that is progressive, varying in intensity and over time can result in severe depletion (1). This condition occurs in disease states such as cancer, congestive heart failure (2), massive burn, sepsis, and major trauma or surgery. The cachectic condition is considered to be a significant factor leading to poor quality of life, high mortality and morbidity rates and poor treatment response in cancer patients (3). Depletion of skeletal muscle tissue is suspected to contribute to shorter survival (4).

In cancer, the prevalence of cachexia varies by the type of cancer, the stage of cancer, age, and sex. The most significant weight loss usually occurs in patients with gastrointestinal malignancies especially pancreatic and gastric cancer (3). In lung cancer patients, the prevalence of weight loss and cachexia is associated with the type of cancer. It has been reported that cachexia affects 60% of non small cell lung cancer (NSCLC) patients (3) and weight loss in lung cancer patients is associated with shortened median survival compared to non-weight losing patients (5). In addition, males with lung cancer experience an eightfold faster rate of weight loss compared to females (6).

Cachexia of disease is generally understood to be characterized by depletion of adipose tissue and muscle mass, and this is in contrast to simple starvation where weight loss is predominantly from fat tissue and skeletal muscle is spared (7). Patients with cancer cachexia suffer from extensive wasting of both adipose tissue and skeletal muscle mass. There is an element of starvation in many patients with cancer and anorexia is a very common symptom among cancer patients. However, investigations on the basic biology and mechanism of cancer cachexia, have shown that limited food intake alone is

not responsible for the wasting of body tissues and when cancer related wasting occurs, muscle tissue is not spared.

The composition of tissue loss, the rate and time of onset of tissue loss, and the intensity of fat and muscle volume change, still is not very clear. Cachexia is thought to involve loss of skeletal muscle and adipose tissue, but it has not been studied longitudinally or in specific muscles or fat depots. Few studies have investigated body composition in the cancer population (8-13), and in most of these studies, clinically expedient and indirect body composition measurements such as skinfold measurement (13), total body nitrogen (12), total body potassium (14), and bioimpedence analysis (15) have been used. None of these methods allow discrimination of individual muscles or of fat depots such as subcutaneous and visceral adipose tissue.

Methods for assessment of human body composition have increased in sophistication with the development of high precision techniques based on imaging technology. These have been used in the evaluation of longitudinal changes and response to various interventions in non-cancer populations, such as in AIDS cachexia (16), or sarcopenia of aging (17). Indirect methods have been replaced by more accurate techniques such as Dual-Energy X-ray Absorptiometry (DEXA) (18), Magnetic Resonance Imaging (MRI) (19), and Computed Tomography (CT) (20). Detailed analyses of CT or MRI images in particular, allow discrimination of cross-sectional areas/volumes of individual muscles and specific fat depots. For example, images of the lumbar area have been used to evaluate subcutaneous and visceral adipose tissue depots (21;22).

Such images exist in routinely acquired medical information of cancer patients in order to follow tumor growth and response to therapy. However these have never been further utilized to evaluate muscle or fat changes. The objective of this work was to conduct a retrospective study of the characteristics of cancer-associated cachexia by studying longitudinal changes in skeletal muscle and adipose tissue in a cohort of patients who died of non-small cell lung cancer. To our knowledge, this is the first study that directly evaluates longitudinal muscle and fat changes of lung cancer patients.

A. Design and Method

A.1. Background: anatomical issues

Background information regarding different types of muscle and adipose tissue is explained in Appendix A.

A.2. Methods and materials

A.2.1. Study design

A retrospective study was designed to determine the rate of wasting in skeletal muscle and adipose tissues in lung cancer patients. A retrospective study is defined as a study based on the medical records of patients, looking back in time at events that happened in the past. A retrospective cohort study uses the records of a specific group of patients (23).

A.2.2. Patient selection

The division of Epidemiology, Prevention and Screening of the Alberta Cancer Board (ACB) operates the Alberta Cancer Registry, a computerized database of all cases of cancer in the province. The registry codes diagnosed primary cancers by their site, morphology, and other biological, clinical and demographic information. The Cross Cancer Institute (CCI) is the only cancer hospital serving Northern Alberta (population 1.8 million) and the majority of all newly diagnosed patients with advanced lung cancer (stage III b and IV) within this zone are treated within this hospital. The design of this retrospective study was based on the opportunity to access this information and the ability to query the database for the cases in question.

In 2001, CCI developed a facility called Picture Archiving and Communication Systems (PACS), which has enabled medical staff to access stored electronic images including X-ray, ultrasound, CT scans, and MRI. The choice of field of study from these archived images was based in part on the prevalence of available CT fields (mainly thoracic, abdominal, head and neck) in the patient record as well as the sites of specific muscles and fat depots. We selected abdominal CT fields including lumbar spinal

vertebrae L2-L4 because of their relative abundance in the patient record, and the fact that this contains visceral and subcutaneous fat, and several muscles: Psoas, Spinal extensors, Transverse and Oblique abdominal muscles and Rectus abdominis. By contrast, the thoracic field lacks visceral fat and the muscle groups tend to be very small, especially in elderly individuals. The initial database query considered all cases and was based on the following inclusion criteria:

-Attended the CCI for various diagnostic and treatment strategies (active treatment, and or palliative treatment).

-Diagnosis of advanced NSCLC (stage IIIb and IV)

-Deceased within 2 years of diagnosis

-Evaluated by CT scan in the abdominal region (see below) at least twice during the interval between diagnosis of advanced lung cancer and death.

-An equal number of male and female subjects were selected, since weight loss in advanced lung cancer has been shown to be sex-dependent (6).

A.2.3. Anthropometric measurements

All the anthropometric data were abstracted from the patient's chart. Weight and height had been measured for each subject at most of the visits to the CCI. Weight was measured using a medical balance beam scale, the subjects had no shoes on and they wore a hospital gown. Weight was recorded to the nearest 0.1 kg. For each subject, height was measured by a stadiometer, without shoes, and was determined to the nearest 0.1 cm. Both measurements had been recorded in the patient's medical chart by a registered nurse. BMI was then calculated. There was no record on the weight and height of one patient at initial assessment due to the patient's condition (inability to stand).

A.2.4. Image analysis

Computed Tomography provides a graphic image of the different tissues (bone, adipose, and muscle tissues) in a scanned area. To calculate the volume and surface, it is necessary to sum the volume/surface of the tissue of interest in serial cross sectional scans of the body.

In this study, regional adipose tissue and non-adipose tissue were measured by CT, which had been performed previously for diagnostic proposes. The third lumbar vertebrae (L3) was chosen as a landmark and four consecutive slices extending from L3 to the iliac crest were assessed to measure volume and cross-sectional area of muscle and adipose tissue. It has been repeatedly reported that visceral adipose tissue (VAT) in a single scan passing through the third to fifth lumbar vertebrae (L3-L5), is a valid predictor of total abdominal fat in males and females (24-28). Since change in VAT was another focus of this study, a separate slice passing through L4-L5 was analyzed to measure this tissue (Figure 1).

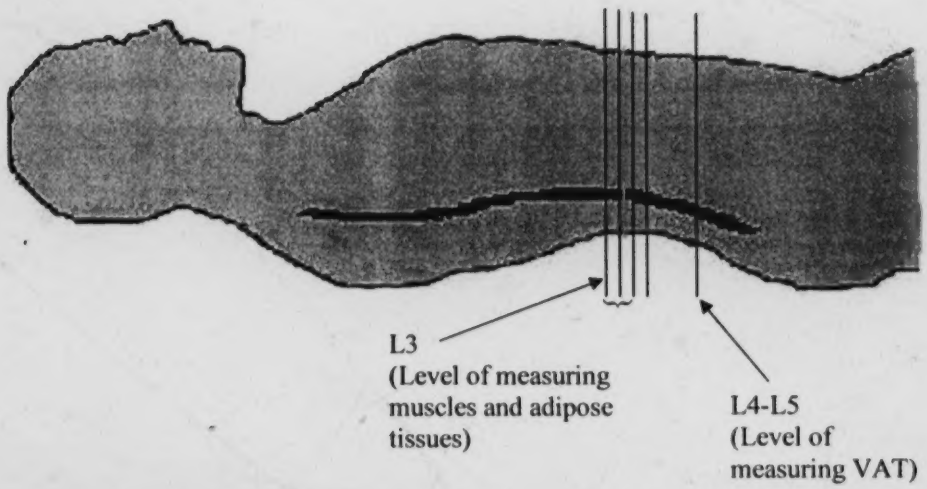

Figure 1. Regional assessment of body composition at abdominal area. Four consecutive slices (6.5 mm thick× 4) starting from L3 were selected to measure muscles and adipose tissues. A single slice (6.5mm thick) at L4-L5 was chosen to assess visceral adipose tissue.

CT scans at the lower abdominal level were taken at various time points for diagnosis or follow up purposes. All the CT data had been obtained with the following exposure parameters: 120 Kvp, 292.0 mA, 512×512 matrix, and 954.81cm^2 (30.9×30.9 cm) field of view. CT images were reconstructed every 6.5 mm. Therefore, each image had 6.5 mm thickness. All the CTs had been scanned and saved in the PACS system, e-film station. At the time of study, selected images were retrieved from the e-film station

and were transferred via DICOM (Digital Imaging and Communications in Medicine) to a personal workstation for analysis using specialized software (Slice-O-matic V4.2, Tomovision, Montreal, QC, Canada). After retrieving each image, the UID number (image identification number) and the image number were recorded. These image identification numbers were required to identify the selected CT images from the Slice-O-matic database.

SLICE-O-MATIC

The Slice-o-matic program is multi-purpose software, which has been designed to visualize and compute the anatomical volumes and surface of different tissues in CT and MRI scans. This software contains three different modules to visualise and calculate the surface and volume including BASIC, TAG, and 3D modules. A combination of the BASIC and TAG modules was used in this study.

A.2.4.a. TAG mode

The TAG module is composed of a group of functions that are required for segmentation, labelling, and computing volume of the tagged anatomical areas.
For this purpose, the program first loads and then displays the CT images. The program only computes the volume if the images are reordered based on their "t" position ("t" is the direction of slices acquisition. For axial slices, where the image is in "x"-"y" plane, "t" would be the "z" axis). After sorting the images, there are six different modes that enable the researcher to segment and compute the images. These are Segmentation, Region Growing, Morpho, Snake, Edit, and Surface/Volume. The first five modes are used to create and edit the TAG files. The Surface/Volume mode is used to compute the anatomical volumes and surfaces identified by the different tag values in the images. In this study initially all images were tagged and computed using the Morpho mode. To confirm the results of the segmentation and to escalate the accuracy of the data, the images were re-examined by Region Growing mode later.

A.2.4.b. Morpho mode

The Morpho mode uses mathematical morphology to segment and edit the image. This mode computes the watershed of the gradient of the GLI (Grey Level Image), which further provides a mosaic pattern of the image. Four different merge options enable the observer to change the size of the mosaic image. Once watershed computation is completed, the next step is to superimpose water parting mesh on the GLI image. This mesh consisted of the set of lines, which defined the various watersheds. Each watershed region must represent only one tissue. When all the watershed regions were determined, each individual region must be painted using a TAG value. At this point, the observer must define a specific TAG value for each tissue. The TAG values used in this study were the following:

TAG 1=red= Spinal extensor muscles

TAG 2= green=Inter-muscular adipose Tissue

TAG 3= dark blue= Oblique/lateral muscle

TAG 4=purple= Rectus abdominis muscle

TAG 5=Yellow=Visceral adipose tissue

TAG 6=Orange=Psoas muscle

TAG 7= Light blue=Subcutaneous adipose tissue

Appendix C demonstrates a colour picture of these tissues.

A.2.4.c. Region Growing/Painting Mode

In this mode, the observer creates the segmented TAG images from the GLI images. To fulfill this purpose, a specific TAG value must be designated to the muscle and adipose tissue respectively. The next step is to select a threshold range for each TAG value. This function is done with the Upper and Lower limit tool. When TAG values are selected for all the tissues under the assessment, one TAG value must be selected at a time. At this point, the paintbrush will precisely tag all the pixels that fall within the range given to the TAG value only.

Selection of the threshold range for each TAG value has a critical importance. In this study, muscle and adipose tissue areas were segmented according to the following standard Hounsfield Unit (HU): skeletal muscle (-29 to +150), subcutaneous adipose tissue (-30 to -190), and visceral adipose tissue (-50 to -150) (20;22;29;30).

A.2.4.d. The Surface and Volume Computation Mode

After each tissue was tagged by Morpho and Region Growing modes, surface and volume was calculated for each TAG region using Surface/Volume Computation mode. However, this mode is unable to calculate the volume if all the images are not sorted according to their t-values. Sorting the images is one of the multiple functions of Admin mode.

To calculate tissue area (cm^2), the surfaces of the respective tissue regions, in each slice, are computed automatically by summing the given tissue's pixels and multiplying by the pixel surface area. For example, the area of different muscle and fat tissues are calculated by summing the area of pixels in each slice with CT Hounsfield unit of -190 to -30 for fat, -29 to 150 for skeletal muscle, according to the method described by Miller et al (22). To compute the volume, the Surface/Volume mode automatically multiplies the surface area covered by the TAG value by the image thickness. In this study all the images had thickness of 6.5 mm. The volume was expressed as cubic centimetre (cm^3). However, where the rate of tissue change was very small, cm^3 was converted to mm^3.

A.2.5. Statistical analyses

This research was conducted as a retrospective study, thus there was no control over the timing of the CT scan and therefore no control over the interval between the two scans for each patient. To accommodate these features of the data set, the date of each CT scan was normalized relative to the date of death, so that scans could be grouped according to whether they took place, for example, within the last 6 months before death or within 6 months to 1 year before death. When the interval between the 2 scans was studied, the change in tissue area / volume occurring over the interval (gain or loss), was divided by the number of days in the interval, to obtain a relative rate of change that

could be compared between intervals and between individuals. The student's t-test and paired t-test were used as the basic test in this study.

A.2.6. Units of data expression

In this study, the absolute volume and the rate of volume change was calculated for each tissue. Table A.2.6.1. shows the units that were used in the results section.

Table A.2.6.1. Description of units used for studying evolution of muscle and adipose tissues in advanced lung cancer patients

Variable	Units	Calculation
Tissue average slice volume in a 6.5 mm thick slice	cm^3	Computed volume of four consecutive slices with 6.5 mm thickness, divided by 4. Used for all tissues except visceral adipose tissue
Single slice volume	cm^3	Computed volume of one slice with 6.5 mm thickness at L4-L5 level. Used for visceral adipose tissue only
Tissue volume change	(gain or loss) cm^3	Absolute tissue change between specified scans ie Table B.1.2
Rate of tissue volume change	(gain or loss) mm^3 / day	Absolute tissue change between specified scans (mm^3) divided by the number of days in the interval between specified scans i.e. Table B.2.3.1
Normalized rate of tissue volume change	% volume change (gain or loss) per 100 days	{(Rate of tissue gain or loss, cm^3 / day) × 100 } ÷ tissue volume (cm^3) at the reference time point i.e. Figure 4, Figure 5

B. Results

B.1. Baseline Characteristics of the patient population

During 2001-2003, a total of 130 lung cancer patients died within two years of diagnosis, and this group included a broad group of ages encompassing 40-85 years and an interval between diagnosis and death ranging from 1-22 months. To narrow the heterogeneity of the patient group, an age interval of 40-80 (median age 60.9±8.6 years) was selected. The median interval between diagnosis and death of the population was 10.1 ± 5.3 months, and thus only those with a 5 to 16 month interval were selected for the study. Not all of the patients who met these criteria had lower abdominal CT scans, and a total of 15 patients were excluded for this reason. Forty-five subjects met all the study requirements (34.6% of 130 subjects). While all of these subjects had at least 2 CT scans, several had 3,4 or 5 scans, such that in total the 45 patients had 113 scans. Figure 2 represents some typical patient histories including dates of diagnosis, CT scans, therapy and death.

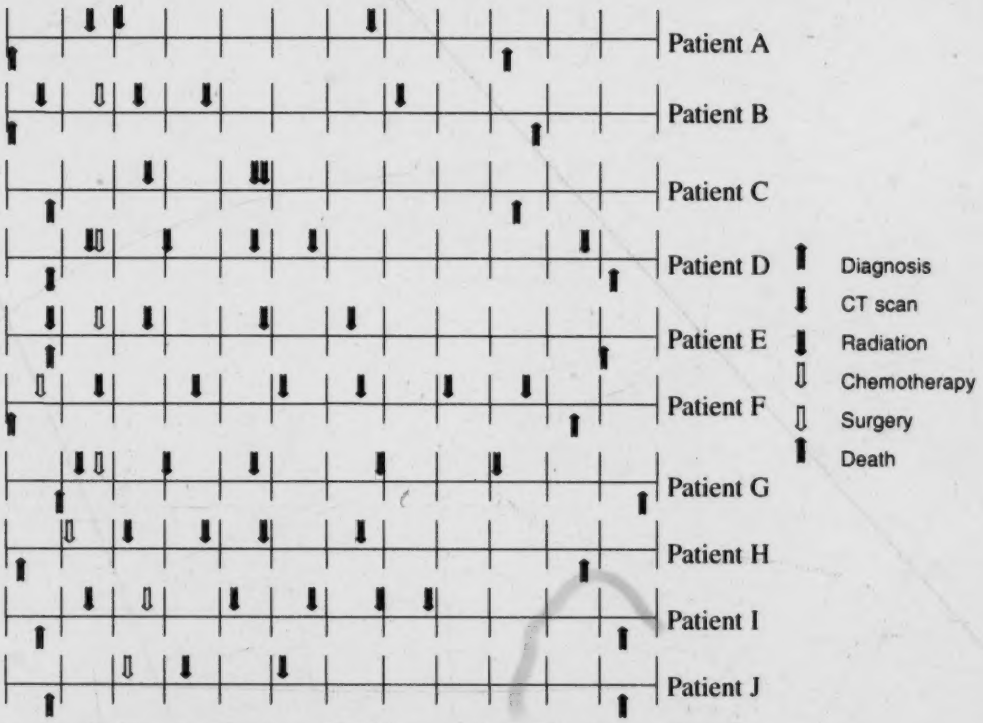

Figure 2. The distribution of CT scans relative to date of death

Each horizontal line represents a year of life of the individual patient, with vertical lines representing monthly intervals. Date of diagnosis (green arrow) and date of death (black arrow) are indicated for each patient. Dates of CT scans, radiation and chemotherapy treatments are indicated. Note that several patients had up to 6 CT scans, covering a large proportion of the interval between diagnosis and death. Some of these are represented in Figures 6-14.

The treatment received by the patient population (n=45) included: no treatment (53%), Chemotherapy (25%), Radiation (9%), Surgery (2%), Chemo-Radiotherapy (9%), and Chemo-Immunotherapy (2%). Of the 45 evaluable scan-scan intervals in the population overall, 21 included the treatment within that window (47%).

All patients had their first CT scan near the time of their first clinical evaluation at the CCI. The first CT scan in the record was on average 221.5 ± 80.2 days prior to the patient's death. The baseline characteristics of the patient population at this time point (first CT scan) are shown in Table B.1.1.

Table B.1.1. Anthropometric and demographic characteristics of advanced lung cancer patients at the time of first CT scan

Variable	Total (n=45)	Male (n=23)	Female (n=22)	P-value[c]	Young (n=20)	Old (n=25)	P-value[d]
Age (year)	60.9±8.7[a] (41-76)[b]	61.0±8.8 (45-76)	60.8±8.7 (41-74)	0.46	53.0±5.1 (41-59)	67.2±4.7 (60-76)	0.00*
Height (m)	1.7±0.1 (1.5-1.9)	1.7±0.1[e] (1.6-1.9)	1.6±0.1 (1.5-1.7)	0.00*	1.7±0.1 (1.6-1.9)	1.7±0.1 (1.5-1.8)	0.13
Weight (kg)	76.4±18.3 (43-123)	84.9±14.6[f] (58.5-123)	66.1±17.3[g] (43-108)	0.00*	73.1±17.1 (48.5-105)	79.1±19.2 (43-123)	0.15
BMI Kg/m^2	26.5±4.8 (18.3-37.1)	27.9±4.1[h] (18.3-37.1)	24.9±5.3[i] (19.1-36.1)	0.03	24.6±4.5 (18.3-32.8)	28.0±4.7 (19.1-37.1)	0.01

[a] Mean ± SD [b] Range [c] P-value for Student's t-test to compare males and females

[d] P-value for Student's t-test to compare young and old (young=40-59 years and old=60-79 years)

[e] one missing data [f] one missing data [g] four missing data [h] two missing data

[i] four missing data *P-value less than 0.01

Because of the well described effects of age and sex on body composition, data for the whole population are stratified by these variables. Males had a significantly greater height, weight and BMI than females, and older patients had a higher BMI. Values for tissue average slice volume at baseline are shown in Table B.1.2. Skeletal muscle volume was greater in males, but was not different between the younger and older patient subsets. Adipose tissue volume was greater in males in both subcutaneous and visceral compartments, and all adipose tissue volumes were greater in the older group. The range of BMI and adipose tissue depots was considerable. Four patients had BMI in the ranges of Class I (30-34.5) and one patient had BMI of Class II obesity (35-39.9) and these had as much as 150-245 cm^3 each of subcutaneous and visceral adipose tissue. By contrast several patients had much smaller fat depots.

Table B.1.2. Tissue average slice volume (cm^3) in abdominal region (L3-L4) in advanced lung cancer patients at the time of first CT scan

Variable	Total (n=45)	Male (n=23)	Female (n=22)	P-value[c]	Young (n=20)	Old (n=25)	P- value[d]
Total muscle	87.5±22.8[a] (55.8 to 155.1)[b]	102.9±21.9 (72.9 to 155.1)	72.3±10.5 (55.8 to 93.1)	0.00*	87.0±23.8 (55.8 to 136.8)	87.9±22.3 (58.5 to 155.1)	0.44
Psoas	12.0±3.7 (6.3 to 23.9)	13.9±4.0 (8.9 to 23.9)	10.0±1.9 (6.3 to 14.2)	0.00*	12.1±4.3 (6.7 to 23.9)	11.9±3.1 (7.9 to 18.3)	0.44
Spinal extensors	36.4±7.1 (24.2 to 54.1)	39.7±7.7 (24.2 to 54.9)	32.9±4.4 (25.1 to 41.0)	0.00*	37.2±8.26 (24.2 to 54.9)	35.8±6.2 (25.1 to 51.1)	0.26
Oblique/Lateral	32.6±11.5 (17.4 to 71.8)	40.2±10.9 (23.3 to 71.8)	24.7±5.5 (17.4 to 37.8)	0.00*	31.0±10.6 (17.4 to 51.6)	33.9±12.5 (19.1 to 71.8)	0.20
Rectus abdominis	6.5±2.7 (3.4 to 14.9)	8.2±2.7 (4.7 to 14.9)	4.7±1.2 (3.4 to 7.7)	0.00*	6.7±2.6 (3.4 to 13.7)	6.3±2.8 (3.6 to 14.9)	0.29
Total Adipose Tissue	179.6±104.4 (3.0 to 411.5)	206.3±100.7 (3.0 to 411.5)	142.4±91.1 (41.3 to 393.5)	0.04	145.5±89.9 (3.0 to 355.5)	209.4±106.4 (47.9 to 411.5)	0.02
Intermuscular	7.1±5.5 (0.1 to 22.8)	8.0±5.8 (0.1 to 22.0)	6.1±5.2 (0.3 to 22.8)	0.12	4.8±4.1 (0.1 to 18.5)	8.8±6.0 (2.0 to 22.8)	0.00*
Subcutaneous	87.3±50.6 (0.0 to 222.2)	99.8±54.0 (0.0 to 222.2)	74.2±46.1 (12.6 to 183.9)	0.04	73.2±44.3 (0.0 to 183.7)	98.6±54.2 (23.6 to 222.2)	0.05
Visceral	85.3±56.5 (2.9 to 245.2)	98.5±51.9 (2.9 to 199.3)	71.4±60.2 (5.0 to 245.2)	0.06	64.4±50.2 (2.9 to 168.8)	101.9±57.76 (16.7 to 245.2)	0.01

[a] Mean ± SD(cm^3) [b] Range(cm^3) [c] P-value for Student's t-test to compare males and females

[d] P-value for Student's t-test to compare young and old (t-test, 1tail, equal variance) *P-value < 0.01

Data are average slice volume (cm^3) calculated as the average of 4 consecutive 6.5 mm thick slices starting at L3 and moving in the direction of the iliac crest.

B.2.Changes in tissue volume during disease progression

B.2.1 Overall changes

The longitudinal changes in tissue volume were determined from the scan – scan intervals. The distribution of CT scans relative to date of death was variable (Figure 1). To capture the overall magnitude of the progressive change, we initially analysed the change in tissue volume between the earliest scan and the last scan that each of the 45 patients had prior to death. The intervals covered by these scans ranged from 40-425 days with the mean of 115 ± 77 days. The average of intervals between first CT and death was 222 ±80 days (range=86-476 days) and last CT to death was 107 ± 57 days (range=22-303 days).

To investigate the absolute tissue change over time, the volume of each tissue was measured at two time points (first and last CT scans) at the lumbar area (L3). The results of this measurement are shown in Table B.2.1.1

Table B.2.1.1 Change in tissue average slice volume (cm^3) in abdominal region in advanced lung cancer patients over time (n=45).

Variable	First CT	Last CT	Δ	P-value
Time to death (days)	221.5 ±80.3[a] (86 to 476)[b]	106.5 ± 57.3 (22 to 303)	115.1 ±77.4 (40 to 426)	0.00* [c]
Total muscle	87.5±22.8 (-6.4 to 31.1)	83.0±20.4 (54.4 to 141.7)	-4.5±7.8 (-6.4 to 31.1)	0.00*
Psoas	12.0±3.7 (6.3 to 23.9)	11.2±3.4 (6.2 to 20.9)	-0.7±1.7 (-7.4 to 1.5)	0.00*
Spinal extensors	36.4±7.1 (24.2 to 54.1)	34.6±6.7 (20.5 to 50.1)	-1.8±3.0 (-12.2 to 5.9)	0.00*
Oblique/Lateral	32.6±11.6 (17.4 to 71.8)	30.9±10.2 (16.1 to 63.4)	-1.7±3.0 (-10.2 to 3.0)	0.00*
Rectus Abdominis	6.5±2.7 (3.4 to 14.9)	6.3±2.3 (3.0 to 12.9)	-0.2±1.2 (-3.7 to2.4)	0.13
Total Adipose Tissue	179.6±104.4 (3.0 to 411.5)	170.9±107.9 (6.9 to 452.3)	-8.7±38.6 (-80.9 to 102.2)	0.11
Intermuscular	7.1±5.6 (0.1 to 22.7)	7.2±5.6 (0.2 to 24.0)	0.2±2.4 (-10.8 to5.5)	0.31
Subcutaneous	87.3±51.1 (0.0 to 222.2)	84.0±53.0 (0.0 to 248.0)	-3.3±16.9 (-39.0 to 42.0)	0.1
Visceral	85.3±57.1 (2.9 to 245.2)	79.7±56.9 (3.8 to 229.8)	-5.6±23.4 (-64.0 to58.3)	0.06

[a] Mean ± SD(cm^3) [b] Range(cm^3) *P-value < 0.01

[c] P-value for paired Student's t-test to compare first and last tissue volume

This analysis, which comprises all cases, shows a significant loss of average slice volume of skeletal muscle tissue, during progressive disease. This affected all of the muscle groups except rectus abdominis, which did not change over time. The loss of spinal extensor muscles, psoas and lateral and oblique abdominal muscles were highly correlated with total muscle. (Table B.2.1.2).

On average, comparing first CT to last CT, adipose tissue volume in the subcutaneous (p=0.1) and visceral site (p=0.06) only showed a small decrease, and this did not reached statistical significance.

It should be noted that for both muscle and adipose tissue, the standard deviation was large and the range included tissue losses (negative values) as well as values that had a positive sign representing gain of tissue during the interval.

The observed changes in the different skeletal muscle tissues were tested for correlations to determine whether the magnitude of change was related.

Table B.2.1.2. Correlation between changes in individual muscles and total muscle change during the interval between the first and last CT scan for each patient

	Spinal extensors	Psoas	Oblique/Lateral	Rectus abdominis	Total muscle
Spinal extensors		0.54[a]	0.18	0.44	0.78
Psoas			0.45	0.29	0.74
Oblique/Lateral				0.43	0.71
Rectus abdominis					0.67

[a] r value for Pearson correlation

The distribution the rate of tissue changes (muscle and adipose tissue) is shown in Figure 3.

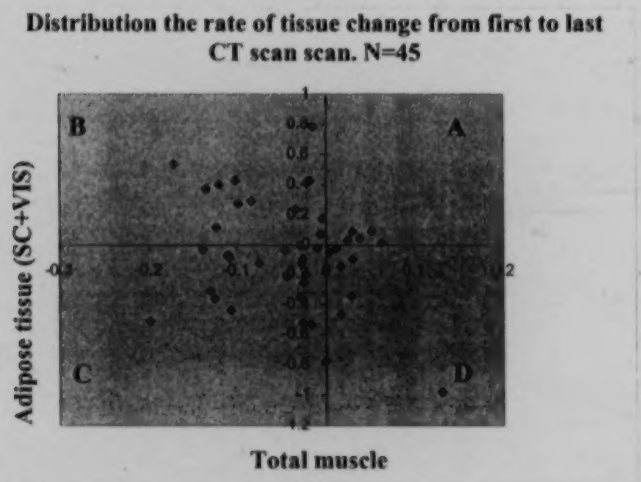

Figure 3. Distribution of muscle and adipose tissue volume changes (cm^3 /day) in all the subjects (n=45). Patients were categorized according to the rate of tissue volume gain or loss (cm^3 /day) for total skeletal muscle and for total adipose tissue in the interval between their first and last CT scans

B.2.2. Tissue evolution at 7-12 months vs 0-6 months before death

As indicated above, the interval between first and last CT scans varied from 40 to 425 days, a rather broad range. To show the course of tissue change in the last year of life with more homogenous intervals, a subgroup of patients were selected, who had one CT in the period 7-12 months prior to their death and the second CT scan in the last 6 months prior to death. Twenty-six of the 45 cases met these criteria. The intervals between these two CT scans were in the range of 60-90 days. The tissue characteristics of this group are shown in Table B.2.2.1. The conclusions reached from this more homogeneous subset of data were almost identical with interpretation of the whole data set, with a significant loss of the three major skeletal muscles and no overall loss of adipose tissue. There was however, a significant increase in intermuscular adipose tissue (p=0.02)

Table B.2.2.1. Change in tissue average slice volume (cm^3) in abdominal region in advanced lung cancer patients during the last year of life (n=26)

Variable	First CT (7.1 months prior to death)	Last CT (4.6 months prior to death)	Δ	P-value
Time to death (days)	211.8±27.3[a] (161 to 254)[b]	138.5 ± 34.6 (78 to 194)	73.5±.20.3 (40 to 136)	0.00*[c]
Total muscle	87.8±20.0 (55.8 to 135.0)	84.4±18.7 (54.4 to 128.3)	-3.4±4.5 (-15.0 to 5.7)	0.00*
Psoas	12.5±4.1 (6.3 to 23.9)	11.9±3.9 (6.1 to20.9)	-0.6±1.1 (-2.9 to 1.5)	0.00*
Spinal extensors	36.9±6.4 (28.6 to 55.3)	35.3±5.9 (27.6 to 50.1)	-1.6±2.0 (-5.9 to1.2)	0.00*
Oblique/Lateral	32.0±9.4 (17.4 to 49.2)	30.8±8.6 (17.2 to 48.2)	-1.2±2.5 (-8.5 to 3.0)	0.00*
Rectus Abdominus	6.5±2.3 (3.5 to 13.2)	6.5±2.3 (3.4 to 13.3)	-0.0±0.8 (-1.9 to 1.4)	0.48
Total Adipose Tissue	177.9±112.[illegible] (3.0 to 393.5)	173.2±110.5 (6.9 to 395.2)	-4.7±33.1 (-69.7 to 88.7)	0.23
Intermuscular	6.3±4.8 (0.06 to 15.3)	6.8±5.3 (0.2 to 18.2)	0.5±1.2 (-2.1 to 4.3)	0.02
Subcutaneous	87.9±48.3 (0 to 174.2)	85.7±48.7 (0.0 to 185.8)	-2.2±17.3 (-44.4 to 32.9)	0.25
Visceral	83.6±66.7 (2.9 to 245.2)	80.6±64.6 (3.8 to 233.6)	-3.0±23.8 (-60.2 to 54.1)	0.26

[a] Mean ± SD(cm^3) [b] Range(cm^3) *P-value < 0.01

[c] P-value for paired Student's t-test to compare first and last tissue volumes

Figure 4 shows the distribution of muscle and adipose tissue changes in this subset of patients.

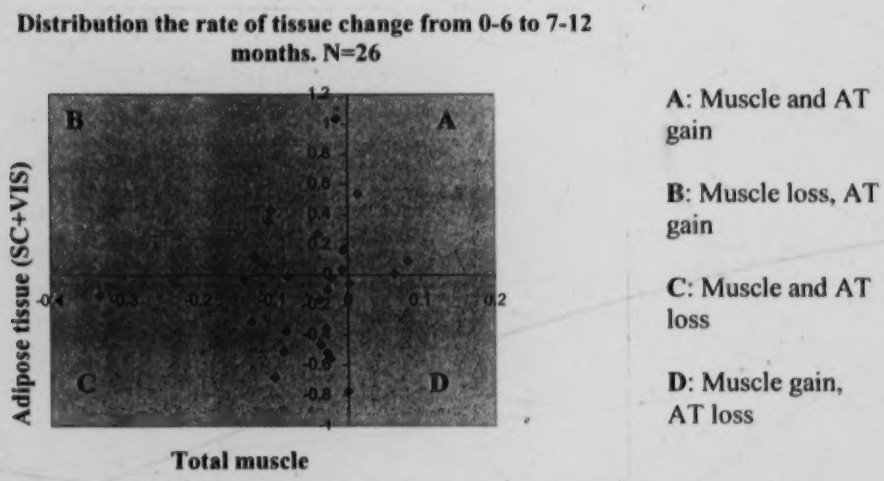

Figure 4. Distribution of muscle and adipose tissue volume changes (cm^3 /day) in last year of life (n=26). Patients were categorized according to the rate of tissue volume gain or loss (cm^3 /day) for total skeletal muscle and for total adipose tissue in the interval between their first and last CT scans

B.2.3. The rate of tissue change

To account for the duration over which tissue changes took place, the absolute change of tissue volume was divided by the number of days in the scan-scan interval. These data are reported as loss or gain in mm^3/day, and represent the rate of tissue change in normalized units. Data in Table 3.2.3.1 represents these results. First the rate of tissue volume change (mm^3/day) for the interval between first and last CT scans was calculated (n=45). Then the rate of volume change was also calculated in last year of life (n=26). These data are concordant with those presented in Tables B.2.1.1 and B.2.2.1 and showed significant loss of the three major skeletal muscle groups, but no overall change in adipose tissue, except for an increase in intermuscular adipose tissue in the subgroup of 26 patients.

Table B.2.3.1. The rate of tissue volume change (mm^3/d) in advanced lung cancer patients over time

	Rate of change from first to last scans (n=45)	P-value[c]	Rate of change in last year (n=26)	P-value[c]
Days from first CT to last CT	115.1±77.4 (40 to 426)	0.00*	70.6±15.1 (40 to 91)	0.00*
Total muscle	-39.6±68.5[a] (-197.0 to 131.3)[b]	0.00*	-53.2±79.7 (-332.6 to 81.7)	0.00*
Psoas	-6.6±15.2 (-49.5 to 18.3)	0.00*	-10.0±18.3 (-57.2 to 16.9)	0.00*
Spinal extensors	-15.6±34.1 (-94.2 to 120.2)	0.01	-22.7±31.6 (-107.8 to 12.9)	0.00*
Oblique/Lateral	-16.6±29.4 (-88.4 to 34.2)	0.00*	-20.3±47.0 (-189.7 to 71.6)	0.01
Rectus abdominus	-1.1±13.8 (-46.3 to 49.5)	0.32	-0.5±14.2 (-46.3 to 22.2)	0.43
Total Adipose Tissue	-67.5±367.4 (-1205.1 to 798.2)	0.11	-69.3±414.4 (-783.5 to 1056.1)	0.2
Intermuscular	0.2±38.0 (-219.6 to 47.7)	0.49	7.1±16.1 (-27.9 to 52.1)	0.01
Subcutaneous	-26.3±182.4 (-431.4 to 327.8)	0.16	-57.6±277.4 (-945.6 to 391.1)	0.15
Visceral	-40.6±216.5 (-554.1 to 455.5)	0.10	-18.9±306.4 (-725.4 to 644.2)	0.37

[a] Mean ± SD(mm^3/d) [b] Range(mm^3/d) [c] p-value from zero difference (no change)

*P-value < 0.01

The rate of muscle and adipose tissue changes in 100 days for 45 patients is demonstrated in Figures 5 and 6.

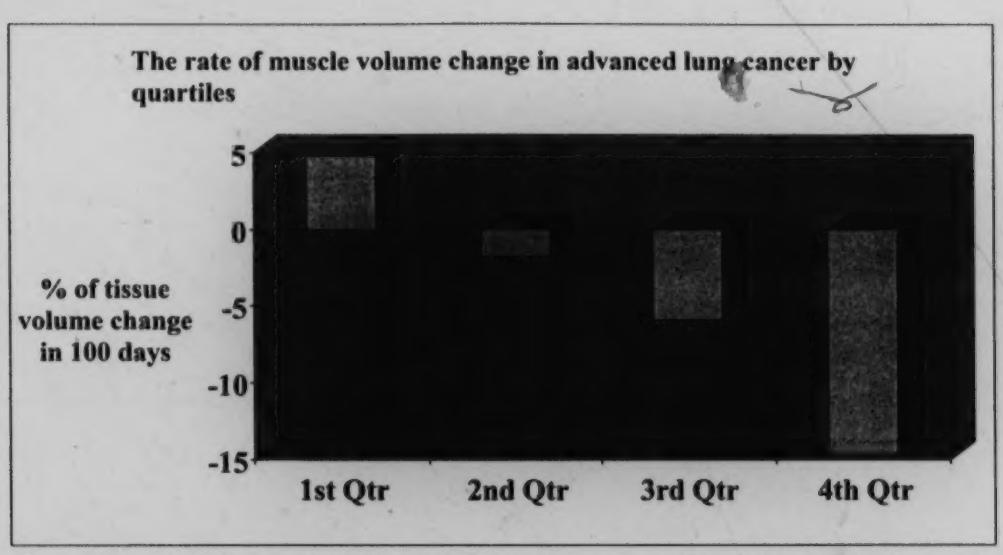

Figure 5. Normalized rate of skeletal muscle volume gain or loss in patients with advanced lung cancer, by quartiles. Data presented in Table B.2.3.1 for 45 patients {the rate of total muscle volume change (mm^3/day)} were recalculated as % volume change per 100 days for each individual and divided into quartiles

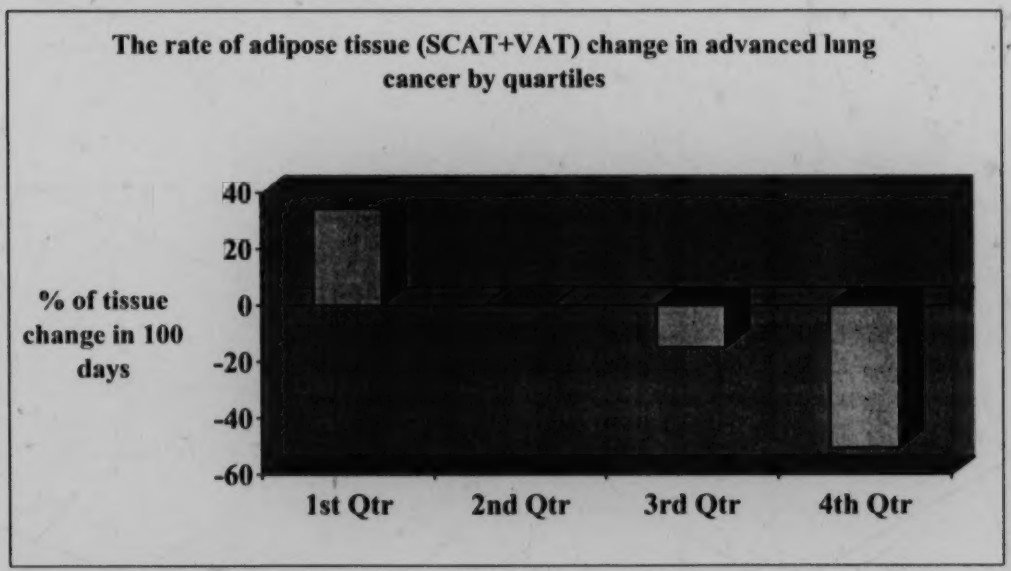

Figure 6. Normalized rate of adipose tissue volume gain or loss in patients with advanced lung cancer, by quartiles. Data presented in Table B.2.3.1 for 45 patients {the rate of total adipose tissue volume change (mm^3/day)} were recalculated as % volume change per 100 days for each individual and divided into quartiles

B.2.4. Detailed progression

A small number of patients in the cohort (n=7) had 4 CT scans. These individual trajectories allow observation of the detailed progression of tissue changes over time. In order to compare these to one another, the initial volume of each tissue was designated to have a value of 100, and subsequent observations were expressed relative to that value (Figure 7-15). The intervals between the CT scans were not identical for all the individuals, however a relatively long period of time (4-11 months) is represented. These figures represent the variation of the responses between individuals. The trajectory of muscle and adipose tissue behaviour could be categorized in three groups of gain, maintain, and loss of tissue. With respect to muscle group, the overall pattern for patient C was a 10% gain in total muscle, which was maintained and this gain was reflected in the behaviour of all the muscle groups. Patients A and G were stable for muscle volume, while patients B, D, E, F tended to lose up to 20% of muscle volume. When the same classification was applied for adipose tissue (Figure 13), it was shown that patients B and C gained a significant amount of total adipose tissue (79% and 14% respectively). Patient A was stable for adipose tissue, and patients D, E, F, and G lost up to 50% adipose tissue. From the group of individuals who experienced muscle loss (patients B, D, E, and F), three patients (D, E, F) lost adipose tissue as well, while one patient (B) gained a very large amount of adipose tissue (79%). The same trend of adipose total tissue change was seen in the behaviour of all the adipose tissue groups.

Once again, these figures confirmed that during the progression of lung cancer, body composition change is different among patients. Some patients may gain both muscle and adipose tissue, some may lose both, and some gain either muscle or adipose tissue and lose the other tissue. However, the majority of patients lost muscle.

Individual trajectories of the detailed progression of tissue changes over time (n=7)

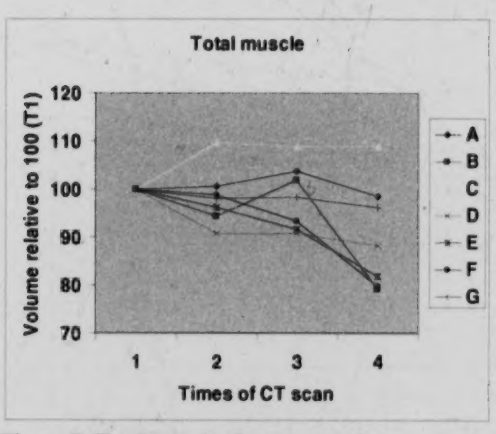

Figure 7. Total muscle change over time

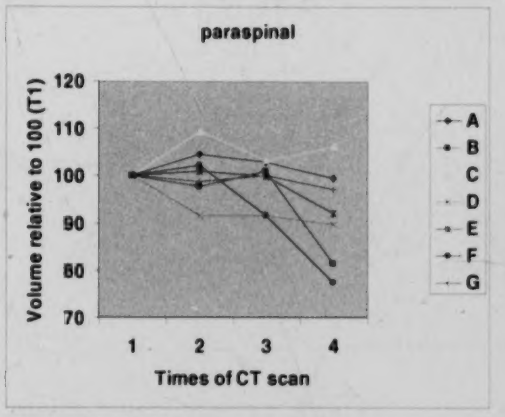

Figure 8. Spinal extensor muscles change over time

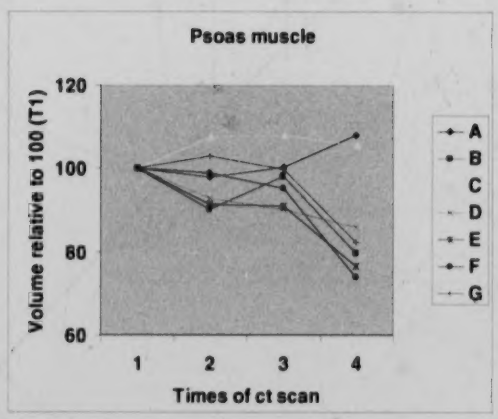

Figure 9. Psoas muscle change over time

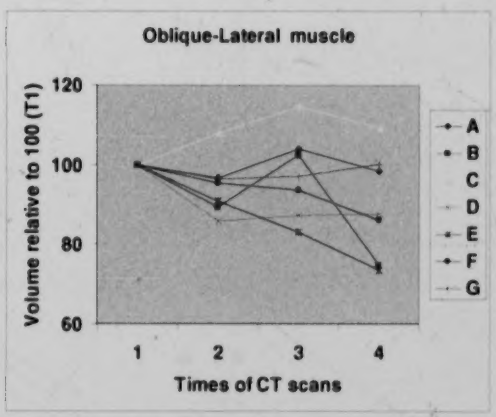

Figure10. Oblique/Lateral muscle change over time

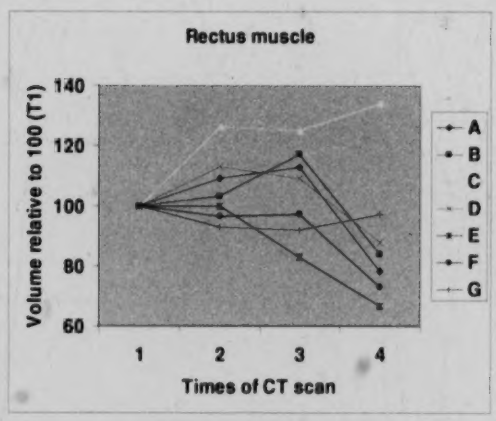

Figure 11. Rectus muscle change over time

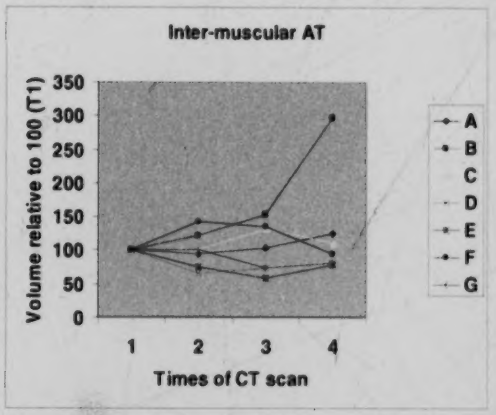

Figure 12. Intermuscular adipose tissue change over time

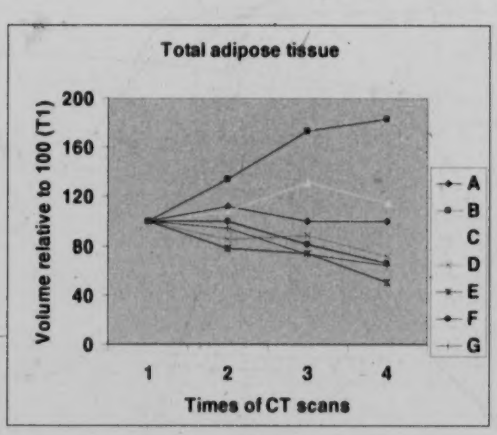

Figure13. Total adipose tissue change over time

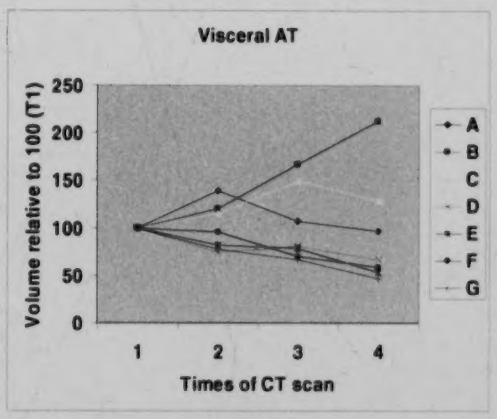

Figure 14. Visceral adipose tissue change over time

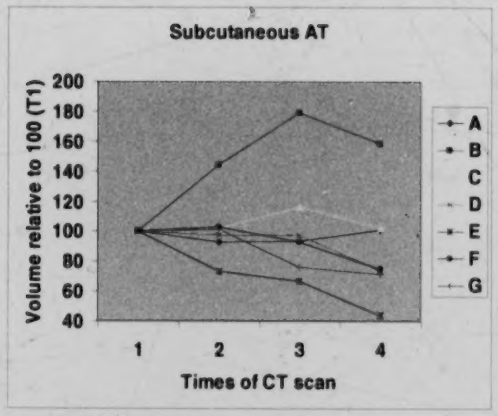

Figure 15. Subcutaneous adipose tissue change over time

B.2.5. Effects of gender

Gender plays an important role in the composition of body tissues. In order to evaluate the role of gender in the change of body composition in cancer patients, all the data were analyzed based on the gender and time of CT scan to death. The demographic characteristics of male and female subjects in this study were shown in Table B.1.1. In this study the cancer population was composed of almost equal numbers of male (n=23) and female (n=22) subjects with a similar mean age (P-value=0.46). However, height, weight and BMI were significantly different between the two sexes (p-value< 0.05). Males were significantly taller and heavier than females. Table B.1.2 shows tissue volumes by gender in male and female subjects. Males had generally larger muscle volume corresponding to their higher BMI, and they also had larger adipose tissue volume.

The next step, to investigate the role of sex, was to compare the rate of tissue volume change in male and female subjects. Table B.2.5.1, and B.2.5.2 show the rate of change in different intervals. Females lost muscle at less than half the rate of males, and for total muscle and the oblique/lateral muscle group this was significant. The percentage contribution of each muscle to total muscle was calculated for males and females. The result of this investigation is shown in table B.2.5.3. Females had a larger proportion of

spinal extensor muscles and a smaller proportion of oblique and lateral muscles and rectus abdominis than males.

Table B.2.5.1. The rate of tissue volume change (mm^3/d) from first to last CT scan in male and female patients with advanced lung cancer

	Male (n=23)	Female (n=22)	P-value
Total muscle	-58.0±73.2[a] (-197.0 to 63.0)[b]	-20.3±58.8 (-138.9 to 131.3)	0.03
Psoas	-8.8±18.1 (-49.5 to 16.9)	-4.4±11.3 (-29.1 to 18.3)	0.16
Spinal extensors	-21.0±26.4 (-88.5 to 12.9)	-9.9±40.5 (-94.2 to 120.2)	0.13
Oblique/Lateral	-24.5±35.9 (-88.4 to 34.2)	-8.5±17.9 (-47.5 to 18.5)	0.03
Rectus abdominis	-3.7±14.2 (-46.3 to 18.8)	1.7±13.0 (-15.2 to 49.5)	0.09
Total Adipose Tissue	-41.7±396 (-783.5 to 798.2)	-87.9±342.3 (-1205.1 to 453.2)	0.50
Intermuscular	6.3±21.3 (-31.7 to 47.7)	-6.2±49.7 (-219.6 to 29.0)	0.1
Subcutaneous	-19.6±192.4 (-357.1 to 327.8)	-33.2±175.6 (-431.4 to 248.7)	0.4
Visceral	-30.5±259.9 (-500.2 to 455.5)	-51.2±165.0 (-554.1 to 179.1)	0.37

[a] Mean ± SD(mm^3/d) [b] Range(mm^3/d)

Table B.2.5.2. The rate of tissue volume change (mm^3/d) in the last year of life in male and female patients with advanced lung cancer

	Male (n=14)	Female (n=12)	P-value
Total muscle	-65.7±99.0[a] (-332.6 to 81.7)[b]	-38.6±49.3 (-138.9 to 14.2)	0.2
Psoas	-12.4±23.2 (-57.2 to 16.9)	-7.3±10.2 (-29.0 to 12.8)	0.2
Spinal extensors	-23.5±33.5 (-107.8 to 13.0)	-21.7±30.7 (-94.2 to 6.8)	0.4
Oblique/ Lateral	-30.4±62.0 (-189.7 to 71.6)	-7.9±13.4 (-27.4 to 8.2)	0.1
Rectus abdominis	0.6±17.4 (-46.3 to 22.2)	-1.7±9.8 (-22.2 to 9.2)	0.3
Total Adipose tissue	-80.5±503.1 (-783.5 to 1056.2)	-56.3±301.4 (-546.8 to 551.0)	0.44
Intermuscular	6.2±12.9 (-13.4 to 34.6)	8.3±19.6 (-27.9 to 52.1)	0.4
Subcutaneous	-133.5±316.4 (-945.2 to 391.2)	31.0±201.6 (-334.0 to 351.9)	0.06
Visceral	46.8±351.0 (-500.2 to 644.2)	-95.6±236.2 (-725.4 to 185.6)	0.1

[a] Mean ± SD(mm^3/d) [b] Range(mm^3/d)

Table B.2.5.3. Percentage of each muscle as a fraction of total muscle volume at L3-L4 in male and female patients with advanced lung cancer

	Total muscle volume (cm^3)	Spinal extensors (%)	Psoas (%)	Oblique and lateral (%)	Rectus (%)
First CT					
Total(n=45)	87.5±22.8[a]	42.4±5.2[b]	13.7±2.4	36.6±4.4	7.3±1.6
Male (n=23)	102.8±21.6	39.3±4.5	13.8±2.8	39.0±3.5	8.0±7.9
Female(n=22)	72.3±10.5	45.6±3.6	13.9±1.7	33.9±3.6	6.5±1.4
Last CT					
Total(n=45)	83.0±20.4	42.4±5.0	13.5±2.2	36.6±4.2	7.5±1.5
Male(n=23)	95.9±19.6	39.5±4.7	13.6±2.7	39.0±3.3	8.0±1.4
Female(n=22)	69.7±10.5	45.5±3.2	13.7±1.6	33.9±3.3	6.9±1.2

[a] Mean ± SD(cm^3) [b] Percentage of each muscle from total muscle volume

B.2.6. Effects of age

Age is another determining factor in body composition. Young individuals have different muscle and adipose tissue composition compared to older ones. In this study, the population age (range 41-76 years) was sub-classified as two groups of young (age < 60 years; n=20) and older patients (age≥ 60 years; n=25). The anthropometric characteristics of these two groups at the time of first CT scan is shown in table B.1.1. Young and older subjects were not different in the proportional contribution of the different muscle groups to total muscle (Table B.2.6.1.).

Table B.2.6.1. Percentage of each muscle as a fraction of total muscle volume at L3-L4 in young and old patients with advanced lung cancer

	Total muscle volume (cm^3)	Spinal extensors (%)	Psoas (%)	Oliique/lateral (%)	Rectus abdominis (%)
First CT					
Total (n=45)	87.5±22.8[a]	42.3±5.1	13.8±2.3	36.6±4.4	7.3±1.6
Young(n=20)	87.0±23.8	43.5±5.2	13.8±2.2	35.1±4.1	7.6±1.4
Old(n=25)	87.9±22.3	41.6±5.0	13.7±2.5	37.7±4.3	7.0±1.7
Last CT					
Total(n=45)	83.0±20.4	42.3±5.0	13.6±2.2	36.6±4.1	7.5±1.5
Young(n=20)	82.1±21.6	43.0±4.8	13.5±2.2	35.7±3.8	7.8±1.5
Old(n=25)	83.7±19.8	41.8±5.2	13.6±2.3	37.3±4.3	7.3±1.5

[a] Mean ± SD(cm^3) [b] Percentage of each muscle from total muscle volume

Young and old subjects were not different in the rate of change of muscle tissues (Table B.2.6.2), however the older subset lost total adipose tissue and visceral adipose tissue with a significantly greater rate than younger subjects. Interestingly, the mean values for all components of adipose tissue change in younger individuals had a positive sign, while these values were negative for the older individuals.

Table B.2.6.2. The rate of tissue volume change (mm^3/d) from first to last CT scan in young and old patients with advanced lung cancer

	Young (53.0±5.1years)[a] (n=20)	Old (67.2±4.7 years) (n=25)	P- value
Total muscle	-47.0±66.3[b] (171.3 to 50.8)[c]	-33.6±71.0 (-197.0 to 131.3)	0.26
Psoas	-9.3±17.5 (-49.5 to 16.5)	-4.5±13.0 (-46.6 to 18.3)	0.14
Spinal extensors	-23.5±32.2 (-94.2 to 20.9)	-9.3±35.0 (-73.3 to 120.2)	0.08
Oblique/Lateral	-13.0±25.4 (-85.0 to 34.2)	-19.5±32.4 (-88.4 to 24.6)	0.23
Rectus abdominis	-2.0±8.7 (-16.9 to 15.2)	-0.29±16.9 (-46.3 to 49.5)	0.33
Total Adipose Tissue	65.3±326.7 (-362.6 to 798.2)	-173.8±369.4 (-1205.1 to 453.2)	0.01
Intermuscular	7.7±16.9 (-22.2 to 47.7)	-5.9±48.4 (-219.6 to 40.8)	0.11
Subcutaneous	12.0±146.1 (-334.0 to 327.8)	-56.9±204.6 (-431.4 to 317.9)	0.10
Visceral	47.2±190.8 (-234.8 to 455.5)	-111.0±213.4 (-554.1 to 221.9)	0.00*

[a] Mean ± SD(years) for age

[b] Mean ± SD(mm^3/d) for tissue volume

[c] Range(mm^3/d)

*P-value < 0.01

B.2.7. Tissue loss versus no tissue loss

As noted above, the behaviour of body composition change was not the same in all patients. This was evident from the large standard deviation of the rate of change of tissues over time and by the fact that the ranges of tissue loss included positive values as well as negative values. Where patients had many CT scans over the course of their illness, it is possible to observe that while some individuals had a characteristic pattern of progressive tissue loss, others showed no change over long periods of time. Several prior

studies have shown that weight loss has a variable incidence in patients with lung cancer, and our data would appear to concur with the presence of a phenotype with muscle loss, fat loss, both or neither. This is illustrated in Table B.2.7.1, where it is shown that 73% of patients lost skeletal muscle during the interval from first to last CT scan (Δ for this interval had a negative sign) and the remaining 27% did not lose or gained muscle.

Table B.2.7.1. Characteristics of the rate of total muscle and adipose tissue volume change (mm^3/d) from first to last CT scan in total population of patients (n=45)

	Tissue Lost	Tissue maintained or gained
Total muscle		
Percentage of total patients	73.3%	26.7%
Rate of tissue change ± SD (mm^3/d)	-67.5±55.1	37.3±33.8
Range (mm^3/d)	(-197.0 to -0.2)	(3.6 to 131.3)
Subcutaneous adipose tissue		
Percentage of total patients	48.9%	51.1%
Rate of tissue change ± SD (mm^3/d)	-168.3±131.0	109.6±103.3
Range (mm^3/d)	(-431.4 to -1.2)	(0 to 327.8)
Visceral adipose tissue		
Percentage of total patients	55.6%	44.4%
Rate of tissue change ± SD (mm^3/d)	-183.4±157.2	137.8±130.5
Range (mm^3/d)	(-554.1 to -0.5)	(0.9 to 455.5)

Among those who lost muscle, 60.6% lost VAT and SC compared to 58.3% AT lost of subjects who didn't lost muscle. Table B.2.7.2 shows the characteristics of this evaluation.

Table B.2.7.2. Percentage of patients who experienced adipose tissue (VAT+SC) gain or loss relative to muscle gain or loss from first to last CT scan in total population of patients (n=45)

	Muscle loss	No muscle loss
Percentage of patients who lost total adipose (VAT+SC)	60.6%	58.3%
Percentage of patients who maintained or gained total adipose tissue (VAT+SC)	39.4%	41.7%

Almost equal proportions of patients lost (49%) or gained (51%) subcutaneous adipose tissue during this interval and 56% lost and 44% gained visceral adipose tissue (Table B.2.7.1.). A sex difference was evident in this distribution, such that a higher proportion of males lost muscle and adipose tissue (Table B.2.7.3).

Table B.2.7.3. Gender-specific characteristics of the rate of total muscle and adipose tissue volume change (gain or loss) from first to last CT scan in patients with advanced lung cancer.

	Male (n=23)		Female (n=22)	
	Lost	Gained or maintained	Lost	Gained or maintained
Total muscle				
Percentage of total patients	78.3%	21.7%	68.2%	31.8%
Rate of tissue change ± SD (mm^3/d)	-84.7±58.4	38.2±14.5	-46.9±44.3[a]	36.7±44.3
Range (mm^3/d)	(-197.0 to -0.2)	(29.2 to 63.0)	(-138.9 to -4.9)	(3.6 to 131.3)
Subcutaneous adipose tissue				
Percentage of total patients	52.2%	47.8%	45.5%	55.5%
Rate of tissue change ± SD (mm^3/d)	-163.9±114.0	137.8±122.1	-173.7±155.2	83.8±79.2
Range (mm^3/d)	(-357.1 to -1.2)	(0.0 to 327.8)	(-431.4 to -9.8)	(2.4 to 248.7)
Visceral adipose				
Percentage of total patients	52.2%	47.8%	59.1%	40.9%
Rate of tissue change ± SD (mm^3/d)	-227.4±158.1	184.2±154.7	-142.8±151.0[b]	81.1±62.7[c]
Range (mm^3/d)	(-500.2 to -19.3)	(0.9 to 455.5)	(-554.1 to -0.5)	(15.7 to 179.1)

a Different from males, p=0.02 b Different from males, p=0.09 c Different from males, p=0.03

Almost identical proportions of young and old subjects showed tissue loss, however the older subjects lost subcutaneous and visceral adipose tissue at approximately twice the rate of younger subjects (Table B.2.7.4).

Table B.2.7.4. Age-dependent characteristics of the rate of total muscle and adipose tissue volume change from first to last CT scan in patients with advanced lung cancer.

	Young (n=20)		Old (n=25)	
	Lost	Gained or maintained	Lost	Gained or maintained
Total muscle				
Percentage of total patients	75%	25%	72%	28%
Rate of tissue change ± SD (mm^3/d)	-73.4±54.1	32.2±14.2	-62.6±57.0	41.0±43.9
Range (mm^3/d)	(-171.3 to -4.5)	(12.4 to 50.8)	(-197.0 to -0.2)	(3.6 to 131.3)
Subcutaneous adipose tissue				
Percentage of total patients	45%	55%	52%	48%
Rate of tissue change ± SD (mm^3/d)	-104.5±106.3	107.5±96.3	-212.5±131.6[a]	111.6±113.6
Range (mm^3/d)	(-334.0 to -1.2)	(0.0 to 327.8)	(-431.4 to -45.6)	(2.4 to 317.9)
Visceral adipose tissue				
Percentage of total patients	50%	50%	60%	40%
Rate of tissue change ± SD (mm^3/d)	-97.3±83.9	191.8±153.0	-240.8±170.4[b]	83.8±77.8
Range (mm^3/d)	(-234.8 to -0.5)	(16.2 to 455.5)	(-554.1 to -19.3)	(0.9 to 221.9)

[a] Different from young, p=0.03 [b] Different from young, p=0.01

B.2.8. Lung cancer patients with loss in muscle and adipose tissue

The data above suggest that fat and muscle loss may be dissociated, at least in some subjects. About 73% lost muscle overall, but only 49% and 56% lost subcutaneous and visceral adipose tissue. Thus, while muscle loss may have been the more generalized phenomenon, at least some of the patients losing muscle tended to be able to retain their adipose tissue. Among cancer patients who were assessed in this study, a subgroup of patients behaved differently from the rest of the sample population. This group, which consisted of eight subjects, experienced weight loss, which was associated with loss in all

the tissues. This exceptional subgroup represented about 18% of the sample size. Patient characteristics are shown in Table B.2.8.1. On average, these patients were initially heavier and had a higher BMI than the overall population.

Table B.2.8.1. Anthropometric and demographic characteristics of patients with advanced cancer who experienced both fat and muscle loss (n=8)

Variable	Total (n=8)	Male (n=5)	Female (n=3)
Age (year)	62±6.45[a] (52-70)[b]	60.6±6.65 (52-69)	64.3±6.65 (57-70)
Height (m)	1.7±0.07 (1.61-1.84)	1.74±0.07 (1.63-1.84)	1.65±0.03 (1.61-1.68)
Weight 1 (kg) at T1	77.9±10.2 (66 to 91)	82.1±10.6 (66 to 91)	71±5 (66 to 76)
BMI 1 (kg/ht^2) at T1	26.7±2.9 (21.6 to 29.3)	27.0±3.2 (21.6 to 29.2)	26.1±2.8 (24.0 to 29.3)

[a] Mean ±SD [b] Range

Table B.2.8.2 shows the rate of volume change for each tissue in this group of patients (mm^3/d). If these values are compared to those for the overall population (Table B.2.3.1.) it is clear that this subset had the most intense and generalized losses. The rate of muscle loss in this subset was 2.6-2.7-fold greater and the rate of adipose tissue loss was 5.4-5.6-fold greater than in the population overall.

Table B.2.8.2. The rate of tissue volume change (mm^3/day) during the course of weight loss in patients who lost both muscle and fat tissue

Variable	Tissue loss (mm^3/day) (n=8)
Total muscle	-104.6±68.0[a] (-197.0 to -23.0)[b]
Psoas	-16.3±14.0 (-46.6 to -3.8)
Spinal extensors	-42.5±30.7 (-82.6 to -9.0)
Oblique/Lateral	-40.8±29.9 (-99.8 to -8.4)
Rectus abdominis	-5.0±15.1 (-23.6 to 16.6)
Total Adipose Tissue	-391.4±202.6 (-678.1 to -38.0)
Intermuscular	-12.3±24.4 (-66.7 to 6.9)
Subcutaneous	-147.7±88.0 (-316.3 to -18.9)
Visceral	-231.5±154.8 (-490.6 to -25.9)

[a] Mean ±SD(mm^3/day) [b] Range (mm^3/day)

C. Discussion

The available dataset was a routine set of clinical information, which was collected not for the purposes of this research, but for evaluation of tumor progression and treatment response. A large fraction of lung cancer patients receive a sufficient number of diagnostic imaging tests, such that it would be practically possible to follow their muscle and fat tissue changes, without having to take additional scans. The value of computer-assisted imaging methods in evaluation of lean and fat tissue change is widely understood, but these methods entail either additional doses of radiation (CT) or access to methods such as DEXA (lower radiation exposure); the latter are not usually found in cancer treatment centres. CT scans, as well as MRI are additionally quite costly and have limited availability. Therefore, it would seem of interest to evaluate these available images, which were taken previously for disease follow up that comprise the standard medical record, and are of sufficient number and quality to also follow body compositional change.

The start and end point of the scan for each patient was dictated by clinical practice, and for these lung cancer patients the vast majority of fields of view were in the thoracic area with approximately 35% of these scans extending to the lumbar area. The lumbar area has been the area of focus of numerous research papers on body composition and composition change in humans because of the presence of key adipose tissue depots as well as several large muscle groups (21;28;31). We justify our focus on the lumbar area because of the larger literature on weight loss and gain providing data on this zone. This is a particularly critical plane for study of visceral and subcutaneous adipose tissue. The skeletal muscles present a different issue. The muscle groups in the lumbar area are largely postural and changes in these muscles may or may not exactly reflect the behaviour of limb muscles used largely for locomotion. The latter explains why a number of studies evaluating muscle loss or gain in elderly or obese subjects focused on the prospective imaging of muscles in the mid-thigh area. Since the main object of oncologic imaging is to follow tumor progression and find the sites of distant metastases, areas of the body such as legs and arms are rarely evaluated using imaging approaches.

The problem that the selective field of view poses, is whether abdominal muscles are representative of changes in muscle in the whole body. One interesting observation that

was made here was that there was a correlation between the spinal extensors, psoas and transverse / oblique muscle volume changes; these muscles whether gaining volume, maintaining volume or losing volume in different individuals, acted in concert. Also the coefficients of correlation of each of these muscles with total muscle volume in the lumbar area were high (B2.1.2). It should be possible to examine different muscle groups in the CT scans of lung cancer patients (ie in the thoracic field the lattissimus dorsi, serratus) to determine whether their behaviour is or is not highly correlated with muscle changes in the abdominal region. For practical utility of using these approaches to follow body composition in lung cancer patients, it would be necessary to identify and validate the use of a specific thoracic skeletal landmark, and muscles and adipose tissue (only subcutaneous) at this level. One constraint on this area is the number of very small muscles, which present problems for accurate quantification. However, if in large part the muscle magnitude and direction of change was consistent for many muscles at different anatomical sites, it would seem likely that the muscle changes could be regarded as systemic effects. In the absence of whole-body images that would allow determination of exactly how muscle volume in any given plane reflected whole –body muscle volume, an analysis of different specific muscle groups would give the best indication of whether the changes were generalized or not.

When evaluating muscle and adipose tissues, correct discrimination of inter-muscular adipose tissue versus muscle tissue may be a problem in these evaluation methods (32;33). Adipose tissue infiltration into muscle lowers its apparent density, so that it may get very close to the range of Hounsfield unit values that are characteristic of adipose tissues. This is most problematic where the lipid is very disseminated in the muscle tissue, as opposed to being present in discreet layers or areas. Examples for this condition are during hemiplegia or under conditions where patients become bedridden, muscles tend to show fatty infiltration (34).

The expression of the data on a volume/day or % change / day basis appears to be the most effective way to compare and to stratify patients within the population as having either no tissue loss in a given interval or having tissue loss of a minor, moderate or high rate (ie assigning a grade to the tissue loss). Although the expression of data as the amount of tissue in mm^3 per day lost or gained is useful, they do not take into account the

very large variation in initial tissue total volume. Therefore, presenting the same data presented as % /day (or %/100 days) of loss or gain of tissue over any time interval can be considered the most useful normalized value.

The major findings of this study were to characterize the change in muscle and adipose tissue during the progression of lung cancer. Muscle loss was the most prevalent and generalized feature of this longitudinal view of progressive cancer cachexia (73% of patients overall) and this was occurring regardless of whether or not fat tissue was being lost.

On one end of the spectrum, 18% of the patients showing muscle and fat loss at a rate of muscle loss (-104.6 mm3/day) that was 2.5-fold higher than the average muscle loss for the whole population (-39.6 mm3/d). In this subset, muscle and fat loss were associated. It would seem likely that in the former case, both negative energy balance and the possible presence of cachectic factors (lipolytic factors, proteolysis-inducing factors) may be at play in causing tissue losses. By contrast, 31.8 to 39.4% of the patients did not lose fat but were losing muscle. In that case, muscle was being selectively lost. It is not possible to comment on what the potential drivers of muscle loss might be, except to state that they might be of humoral origin (ie cytokines) of tumor origin (PIF), related to lack of contractile activity, or to lack of anabolic stimulus (ie testosterone) (8;35-37).

It is perhaps of most particular interest that one quarter (26.7%)of patients did not lose any muscle at all and that for some patients this lack of muscle loss was documented in up to 4 scans over an extended period of many months. This methodological approach allows for a very precise classification of muscle loss rate, which can be practically applied in the clinic. For example, Simons et al. compared weight-losing versus non-weight-losing male lung cancer patients matched for age and disease stage (8). It should be possible using these approaches to classify and then compare patients in the highest and lowest quartiles for muscle loss. This would be a powerful comparison if patients were matched for age, sex and disease stage and would serve to isolate those factors associated specifically with muscle loss. In this study, forty-five consecutive patients who died of NSCLC were evaluated for loss of abdominal muscle during their advanced disease trajectory. In this retrospective study, the 1st CT scan evaluated took place on average at 225 days before the date of death and the 2nd evaluated scan took place on average at 107 days before death. Total abdominal muscle

loss or gain was expressed as % change over the scan-scan interval, and these values are expressed as a rate of % change per 100 days. Overall, for all patients combined, muscle change was -4.5±7.8%/100 d and this was significantly different from zero. However, the large standard deviation is reflective of the wide variation in this population, and this is further revealed by dividing the population into quartiles. Note that the top quartile of these patients maintained or even gained a small amount of muscle tissue (+4.6 ± 1.27%/100d), while patients in the 4th quartile lost 14.6 ± 1.99 % of muscle volume/100 days. This analysis not only provides an accurate estimate of muscle volume, but also allows for characterization of the dynamic state of loss.

When adipose tissues were assessed, Fat loss and fat gain were almost equally prevalent, such that overall there was only a trend ($p=0.06$ to $p= 0.11$) for fat loss and this could only attain significance with a much larger sample size than the present study. The unexpected result of this study was the observation of fat gain in visceral and subcutaneous compartments that was, in some patients, substantial. This is however, not so surprising if one takes into consideration that energy intake may be maintained or even increase when energy expenditure falls. Caloric intake might improve with some dietary consultation, upon alleviation of symptoms such as nausea or pain that may inhibit food intake, or upon treatment with corticosteroid. At the same time, patients with cancer may have very low physical activity (38), especially if they become bedridden. It has been previously shown that increases in energy intake induced by glucocorticoids and by progestational agents in cancer patients result in fat deposition. This does not occur in muscle, possibly because there is no particular anabolic stimulus or environment for muscle or lean tissue deposition. Fearon et al. and Lundholm et al. achieved weight maintenance with advanced cancer patients by increasing dietary intakes from 24 to 34 kcal/kg BW/ day (39) (40).

While fat loss and intense fat loss is not a surprising observation, it will be necessary to examine more closely the possible causes, of which low caloric intake and specific lipolytic factors are the most probable. Low energy intakes are endemic in this population due to a large variety of factors: such as anorexia, nausea, vomiting, delayed gastric emptying, and depression.

Similar to muscle loss, quartile classification was applied for adipose tissue behaviour in the total population of 45 patients. Almost 50% of the population were able to gain or maintain their adipose tissue. In the highest quartile, patients gained up to 34% of adipose tissue in 100 days. In the second quartile patients almost maintained adipose tissue (0.6% gained in 100 days). For the last two quartiles of the population, the rate of adipose tissue lost were -15%/100 days, and –50.1%/100 days.

When weight loss due to diet and exercise occurs, visceral adipose tissue is mobilized to a greater extent than subcutaneous adipose tissue (41;42). When visceral adipose tissue was assessed by gender, this study reached the same conclusion as others (28) that visceral adipose tissue volume is greater in males compared to females (P=0.06). Examining this parameter in the young and older group showed that older subjects have more visceral adipose tissue than young subjects (p=0.01). Also, the rate of wasting for VAT was significantly higher in the older group (p=0.00)

Sources of variation

In studying body composition in any condition, numerous variables play a role, such as gender, age, dietary intake, treatment, and physical activity. Some of these factors have been highlighted in chapter two. Due to the retrospective feature of this study, assessment of some of these variables such as dietary intake and physical activity was not possible. Although treatment information was available through the patients' medical chart, in most cases, treatment did not match with the time of the CT scans. Therefore, among these factors, only gender and age were evaluated in this study.

Gender: Gender is an important factor in determining body composition. In this study, male subjects were taller and heavier than females; males also had more muscle and adipose tissue (subcutaneous and visceral) volume than female subjects. Comparing the percentage of each individual muscle as a fraction of total muscle showed that females had bigger percentage of spinal extensors muscle compared to male subjects. Loss of muscle was especially prominent in males: a higher percentage of male subjects lost muscle (males78% vs females 68%) and subcutaneous adipose tissue (males 52% vs females 45%). In addition, male subjects tended to lose muscle almost 2.5 times faster

than females. However, comparing the rate of adipose tissue wasting by gender did not show a major difference (41.7 vs 37.0 mm^3 /d).

Other literature supports a differential prevalence and rate of weight loss in male and female lung cancer patients, and the present analysis demonstrate the gender-specific character of muscle and adipose tissue loss. It is not presently possible to explain this gender difference. Regarding gender and age differences in adipose tissues, the degree of variation in adipose tissue volume and rate of change was so great, that it precluded clearly defining some of the possible changes (ie p= ~ 0.1).

Age: The age range for the patients in this study was from 40 to 80 years old. To investigate the role of age, patients were categorized into two age groups of young (40-59 years old) and old (60 to 80 years old). Each group comprised 50% of the population. The first CT scan was used to compare body composition of these two proposed groups. The results showed that the main difference is in adipose tissue, especially in VAT(P=0.00). With respect to muscle change, both young and old lost total muscle similarly. But when each muscle was taken into account, spinal extensors was the main muscle, which was lost more vigorously in the young (P=0.08) (B.2.6.2)

Unknown variation: There are a number of suspected causes of variation in cancer cachexia. By its nature as a retrospective study, this information is not currently available for the patient group studied here, and in fact the relevant information (dietary intakes, metabolic rate, physical activity, profile of anabolic and catabolic factors) usually is not in the patient record. Prospective studies will be required to identify the relation between these factors and muscle and fat changes.

In summary, this study showed that the majority of lung cancer patients, regardless of age and gender, are susceptible to loss of muscle tissue in the abdominal area. However, the incidence of adipose tissue loss, in this region of the body, is not as common as muscle wasting in this group of patients. In this study, muscle, intermuscular, and subcutaneous adipose tissues were assessed using the average of four CT scan slices extending down from L3, and visceral adipose tissue was evaluated using a single slice at L4-L5 level. Findings of this study may raise a question as to whether regional study of

body composition can be used to assess whole body composition. Recently Shen et al. (43) conducted a study to answer this question. Their results showed that there is a significant relationship between abdominal skeletal muscle and adipose tissue from a single image at lumbar area and total body muscle and adipose tissue. Therefore, we expect that the findings of this study can be applied for total body composition. However, further investigations are required to support this hypothesis.

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Chapter Four

Prospective assessment of body composition of patients with advanced cancer by using DEXA

Introduction

Investigation of body composition has been an important area of human research for many years. It is well documented that some life-threatening conditions are accompanied by body composition changes, which may influence the patient's survival and quality of life. Congestive heart failure, AIDs, extensive burns, head injury, Anorexia Nervosa, surgery, dialysis, and cancer are examples in this category. Knowing the trajectory of body composition changes in these situations and initiation of appropriate interventions is believed to be important to lower the rates of morbidity and mortality. Researchers have developed and applied different methods to study the course of change of different body tissues in various diseases and conditions. Although many methods are available, only a few of them are sufficiently accurate to evaluate fat and muscle mass in clinical research. Generally, these measures are categorized into four groups: anthropometric, biochemical, bioimpedance/resistance and electroconductivity, and imaging techniques. Characteristics of many of these methods have been previously discussed (Chapter Two).

At present, the most accurate methods for measuring adipose tissue and muscle tissue are imaging techniques which include multi-scan computerized axial tomography(CT), magnetic resonance imaging(MRI), and dual energy X-ray absorptiometry(DEXA). The ability of these methods to precisely measure regional and whole body muscle and adipose tissue distribution, make them the primary methods.

Although CT and MRI represent advanced technology in evaluating body tissues, there are some obstacles that limit their use in research. Increased expense, lack of access to imaging facilities as well as the radiation hazard of CT scanning has limited their widespread use and acceptability. In contrast DEXA is a gold standard alternative to measure body compartments with its lower cost and lower radiation exposure.

Two decades ago, Peppler et al. (1) introduced dual photon absorptiometry (DPA) as a way to measure bone mineral and lean body mass. When other researchers compared DPA with neutron activation methods to measure total body calcium in ten subjects, results showed that although the correlation between two methods was more than 0.99, the radiation exposure of DPA was 500-5000 times smaller than neutron activation (2). In the early 1990s, DPA was substituted with its advanced model called DEXA.(3;4). In this advanced method, the radionuclide source of the photon was replaced with an X-ray source. Ever since, DEXA has been used as a gold standard tool to measure body compartments in various diseases including AIDs (5;6), cancer (7), hemodialysis (8), and age related sarcopenia (9).

Cancer is a worldwide devastating disease. In Canada, for the year 2004, it is estimated that there will be 145,500 new cases and 68,300 deaths from cancer (10). The overall prevalence of cancer in Canada is 2.6% and 2.4% respectively in females and males(10). One of the key features of this disease is depletion of host reserves of adipose tissue and skeletal muscle mass, a condition, which is known as cancer cachexia.

Approximately half of the cancer patients experience the cachexia syndrome, which has significant negative impact on the quality and duration of life of cancer patients. The contribution of cachexia as a major cause of death in cancer varies from 1% to up to 20% between studies (11;12). Despite the controversy of the role of cachexia in cancer death, it is clear that patients who experience cancer cachexia are at higher risk for morbidity and mortality. The rate and quantity of tissue loss may be key prognostic indications.

The purpose of this pilot study was to evaluate the rate of muscle and fat changes in advanced cancer patients and also to investigate the relationship between nutritional intake and body composition changes.

A. Design and Methods

A.1. Research Design and Patient Recruitment

The present study was conducted at the Cross Cancer Institute (CCI), Edmonton, Alberta. The research was designed as a longitudinal study comparing body composition and dietary intake of advanced cancer patients over a period of 4-6 weeks. Patients were

considered as their own control. This specific group of cancer patients was selected because cachexia is a common complication in this population and to the best of our knowledge no study has examined their body composition over time.

Subjects were recruited from two specific patient groups. These included palliative patients who were receiving palliative care at the Pain and Symptom Clinic located at CCI; and patients who were receiving active treatment for progressive disease or those who were being followed by their attending physicians at the Out Patient Clinics located at CCI. Participants included in the study had an advanced form of cancer (Metastatic disease) and a history of progressive weight loss in the previous six months. A sample size of n=20 was proposed for the study.

A.2. Methodology

Body composition measurement and dietary record analysis were the two main methods used to meet the goals of the study.

A.2.1. Body composition Measurements: Weight and Height

Weight and height were measured for each subject at the Imaging Centre, prior to DEXA scanning. Weight was measured using a medical balance beam scale (Health O meter) while subjects had no shoes on and they wore a hospital gown. Weight was recorded to the nearest 0.1 kg. Standing height was measured to the nearest 0.1 cm with a stadiometer. All of these measurements were entered as the patient's demographic information in the DEXA report.

A.2.2. Body Composition Measurement: DEXA

Body composition was measured on two occasions, at the beginning of the study: time one (T1), and 4-6 weeks later: time two (T2). All of the body composition measurements were performed at the same location by two trained technicians using standard procedures. For patient comfort, in most cases, DEXA appointments were made on the same day as their appointment at CCI.

Metal objects interfere with the measurement of BMD (Bone Mineral Density). Thus, all patients were asked to wear the hospital gown and remove metal objects such as jewellery. Each patient was required to lie flat in a supine position on the x-ray table without a pillow. The technician positioned the patient's body straight on the mat, relative to the centre lines. Patient arms were placed at their side, with palms down and separated from the thighs. The patients were asked to remain still during the scanning process. A series of transverse scans from head to toe were performed for each DEXA, with a scan speed of five cm/sec. The total time for the DEXA scan was approximately four minutes.

Fat mass, lean soft tissue and bone mass were measured at each time point by DEXA using a total body scanner (model QDR 4500A; Hologic Inc. Version 11.2). The Hologic QDR 4500 uses a low level of x-ray. According to the manufacture's manual, when the whole body is scanned, the scan mode is fast and under standard operating conditions, the entrance dose of radiation to the patient is 1 mR (milliroentgens) (compared to a radiation dose exposure in an standard chest x-ray that is approximately 35 mR). In addition, the radiation exposure at a distance of two meters from the equipment is less than 1mR/hour. Therefore, no additional shielding was necessary for the patient, and the operator. The QDR 4500 includes a multi-element detector array and fan-beam geometry for performing high speed scans.

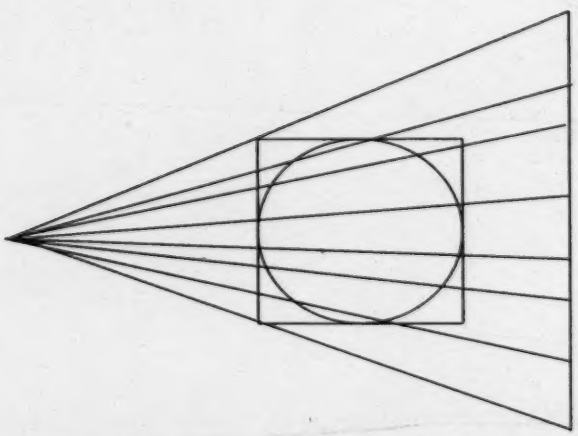

Figure.1. Array mode scanning with fan-beam geometry

Upon completion of scanning, the system automatically proceeds to analysis mode.

A.2.3.Nutritional intake

Dietary intake was measured using three-day food records at T1 and T2.Subjects were instructed to record all food and beverages that they consumed for three consecutive days (one weekend and two weekdays). The amount of all food and beverage consumed was recorded in household measures. In addition, the method of meal preparation and the time of food intake were recorded. Dietary intake was quantified by frequency of daily meals including beverages and snacks. Intake of energy and nutrients (macro and micro-nutrients) was calculated using a computerized nutrition software program (Food Processor, version 6.0, ESHA Research, Salem, OR). This software uses a database of over 10,000 food items from a variety of food groups, including fast food and frozen food, which are common and available in Canada and the USA. This program provides nutritional information for over 113 nutrients.

Food records are a widely used method for measuring energy and nutrient intake in nutritional research. Validity and reliability of the dietary food records have been previously investigated (13;14). In general, the results indicated that the food records usually result in underestimates of energy intake (15).However, when main nutrient intakes obtained from food diaries were compared to those obtained from observed intake, results for most nutrients were not significantly different between methods (16).

A.2.4.Statistical Evaluation

Body composition was followed over a 4-6 week period. To standardize the observations for individual patients, each measurement was expressed as a percentage change from the value at T1. To conduct statistical analysis, the software program, and Statistical Package for the Social Science (SPSS, version12.0) was used.

Analysis of the data was conducted using paired t-tests to compare body composition and food intakes at T1 and T2 and Pearson's correlation coefficients to examine the relation between body composition change and dietary intake. A P value <

0.05 was considered significant. All the values in the text, tables, and graphs are expressed as the mean ± SD.

A.2.5. Ethical Approval

The study protocol and methods were approved by the Research Ethics Board, Alberta Cancer Board and panel B, Health Research Ethics Board, Capital Health Authority. All subjects provided written consent before participation.

B. Results

B.1. Subject recruitment and final sample size

This study was designed as a pilot study requiring a sample size of n=20 subjects. However, over the period of sixteen months, only nine patients volunteered to participate. Of these participants, three died before completing the study, and one subject withdrew from the study due to her debilitating condition. Thus, a total of five patients successfully completed the two DEXA measurements and of those, only four were able to complete three-day food records. Among these nine patients, four had gastrointestinal(GI) malignancies, three had lung cancer, one suffered from squamous cell carcinoma (SCC) of the nasal cavity, and one patient was diagnosed with breast cancer. All the patients had either GI or brain metastasis at the time of participating in the study. The patients' results have subsequently been analyzed and presented as a case series. A case series study refers to a report of observations on more than one subject with the aim to identify important objectives. This type of study is mainly used to generate hypotheses for future studies. Although the results obtained from a case series study suggests association between variables, one can not conclude a cause and effect relationship(17).

B.2. Subjects characteristics

Weight, height, body mass index (BMI), and percentage of body weight change were calculated from the anthropometric measurements performed at the time of the DEXA scan. Pre-illness weights for all patients were obtained from their medical charts.

Anthropometric and demographic information for each patient is shown in Table B.2.1.

Table B.2.1. Anthropometric and demographic characteristics of all the patients with advanced cancer.(n=9)

Patient #	Height (m)	Pre-illness weight (kg)	Weight 1 (kg)	Weight 2 (kg)
1	1.61	63.6	48.3	52.1
2	1.76	68.0	88.0	N/A[a]
3	1.63	50.0	42.5	44.0
4	1.60	58.0	47.5	N/A[b]
5	1.75	77.0	78.0	N/A[b]
6	1.61	67.0	60.5	N/A[b]
7	1.87	73.0	65.0	56.6
8	1.77	76.0	53.5	48.0
9	1.64	73.0	65.2	61.3

[a] withdrew from the study after first DEXA and dietary record

[b] died before second DEXA and dietary record

Table B.2.2. demonstrates the average of anthropometric characteristics of all patients and different genders.

Table B.2.2. Anthropometric and demographic characteristics of advanced cancer[a] patients evaluated by DEXA (T1) (n=8)

	Females (n=3)	Males (n=5)	Total (n=8)
Age (year)	51.3±4.5[b]	54.8±7.5	53.5±6.4
Height (cm)	161.2±1.2	172.8±10.5	168.5±10.0
Weight (kg) (T1)	46.1±3.1	64.4±8.9	57.6±11.8
BMI (kg/m^2) (T1)	17.8±1.5	21.7±3.7	20.3±3.6
Pre-illness weight (kg)	57.2±6.8	73.2±3.9	67.2±9.5

[a] Cancer types in these patients included nasal (n=1), GI (n=4), breast (n=1), and lung cancer (n=3)
[b] Mean ± SD

For eight patients, the age was in the range of 44-56 years. Only one patient was 65 years old. Therefore for all the subjects the mean age was skewed to 53.5 years. The range of weight at T1 was 42.5 to 78 kg with a mean of 57.6 kg and BMI range was from 16.1 to 25.5 with a mean of 20.3. The anthropometric characteristics at T1 and T2 of five patients who completed the study are shown in Table B.2.3. The interval between T1 and T2 was 49.2±9.6 days.

Table B.2.3. Anthropometric and demographic characteristic of patients with advanced cancer at T1 and T2 (n=5)

	Females (n=2)	Males (n=3)	Total (n=5)
Age (year)	51.5±6.4[a] (47-56)[b]	51.7±6.7 (44-56)	51.6±5.7 (44-56)
Height (cm)	161.6±1.4 (160.6-162.6)	176.0±11.7 (163.7-186.9)	170.2±11.4 (160.6-186.9)
Pre-illness weight (kg)	56.8±9.6 (50-63.6)	74.0±1.7 (73-76)	67.1±10.6 (50-76)
Weight 1 (kg)	45.4±4.1 (42.5-48.5)	61.2±6.7 (53.5-65.2)	54.9±10.1 (42.5-65.2)
Weight 2 (kg)	48.1±5.7 (44-52.1)	55.3±6.7 (48-61.3)	52.4±6.8 (44-61.3)
BMI 1 (kg/m^2)	17.4±1.9 (16.1-18.7)	20.0±3.9 (17.0-24.3)	18.9±3.2 (16.1-24.3)
BMI 2 (kg/m^2)	18.4±2.5 (16.62-20.19)	18.1±4.2 (15.3-22.9)	18.2±3.2 (15.3-22.9)
Weight change (%)[c]	5.7±3.1 (3.5 to 7.9)	-9.7±3.5 (-12.9 to –6.0)	-3.8±8.9 (-12.9 to7.9)

[a] Mean ± SD [b] Range
[c] percentage of weight change from T1 and T2

Weight change between T1 and T2 varied among subjects, ranging from 7.86% gain in one female to -12.93% loss in one male. According to the percentage of weight change, unlike females who tended to gain weight, males were susceptible to weight loss.

B.3. Body Composition (DEXA)

Body composition was measured by DEXA. Body composition measurement for each patient is shown in Table B.3.1.

Table B.3.1. Individual baseline evaluation of body composition in patients with advanced cancer determined by DEXA[a] (n=9)

Patient #	Fat mass (kg)	Lean mass (kg)	Fat %	BMC (kg)	BMD(g/cm^2)
1	5.23	42.23	10.6	2.12	1.17
2	12.30	77.88	13.3	2.27	1.08
3	12.24	32.11	26.3	2.10	1.14
4	9.98	36.44	20.7	1.85	1.05
5	21.06	56.29	26.4	2.44	1.10
6	10.64	49.15	17.2	2.16	1.10
7	9.54	53.27	14.5	2.90	1.16
8	5.17	46.56	9.5	2.75	1.30
9	17.56	46.34	26.5	2.44	1.15

[a] Dual Energy X-ray Absorptiometry

Values measured at T1 for females and males and the total group (n=8) are shown in Table B.3.2.

Table B.3.2. Baseline evaluation of body composition in patients with advanced cancer determined by DEXA[a] (n=8)[b]

	Females (n=3)	Males (n=5)	Total (n=8)
Age (year)	51.7±4.0[c] (48-56)[d]	54.8±7.5 (44-65)	53.6±6.3 (44-65)
Fat mass (kg)	10.0±3.6 (5.2-12.2)	12.8±6.4 (5.2-21.1)	11.4± 5.5 (5.1-21.1)
Lean mass (kg)	36.4±5.1 (32.1-42.2)	50.3±4.4 (46.3-56.3)	45.3±8.1 (32.1-56.3)
Fat (%)	19.2±8.0 (10.6-26.3)	18.8±7.5 (9.5-26.5)	18.9±7.0 (9.5-26.5)
BMC[e] (kg)	1.9±0.2 (1.9-2.1)	2.5±0.3 (2.2-2.9)	2.3±0.3 (1.9-2.9)
BMD[f] (g/cm^2)	1.1±0.1 (1.1-1.2)	1.2±0.1 (1.1-1.3)	1.1±0.1 (1.1-1.3)

[a] Dual Energy X-ray Absorptiometry

[b] One female patient was excluded from study due to the presence of huge edema and ascitis

[c] Mean ± SD [d] Range [e] Bone Mineral Content [f] Bone Mineral Density

For all subjects, fat mass ranged from minimum of 5.2 kg to 21.1 kg with mean of 11.4 kg. The range was 32.1-56.3 kg and mean for lean mass was 45.3 kg. Respectively, fat percentage ranges from 9.5 % to 26.5%. In addition to fat and lean tissue mass, bone mineral content (BMC) and bone mineral density (BMD) are two other parameters that are measured by DEXA. BMC expresses bone mass, whereas BMD represents the compactness of bone. Values for BMC and BMD ranged between 1.9-2.9 kg and 1.1-1.3 g/cm^2 respectively. Data showed that the average values for fat mass and lean mass were consistently higher in males compared to female subjects.

Body composition changes over 4-6 weeks were calculated for those patients who completed the study (n=5). The students paired t-test was used to compare the tissue mass values for each tissue. The results are shown in Table B.3.3. Although none of the changes met the required p value (P value < 0.05), fat mass percentage change was approximately 0.06, which was closed to the significant P value of < 0.05 and a trend was observed for BMC (P value =0.09).

Table B.3.3. Comparison between body composition measurement at T1 and T2 for all patients (n=5) and different genders (males and females)

	Females (n=2)			Males (n=3)			Total (n=5)		
	T1	T2	P-value	T1	T2	P-value	T1	T2	P-value
Fat mass (kg)	8.7±5.0[b]	8.2±3.3	0.36	10.7±6.3	8.5±5.4	0.07	9.9±5.2	8.4±4.2	0.06
Lean mass (kg)	37.2±7.2	39.2±9.0	0.18	48.7±3.9	45.2±3.3	0.04*	44.1±7.8	42.8±6.1	0.22
Fat (%)	18.5±11.1	17.2±8.7	0.29	16.8±8.7	14.6±7.8	0.12	17.5±8.4	15.6±7.1	0.06
BMC[c] (kg)	21.1±11.9	20.9±55.9	0.27	2.7±0.2	2.6±0.2	0.14	2.5±0.4	2.4±0.3	0.09
BMD[d] (g/cm^2)	1.2±0.0	1.1±0.1	0.01*	1.2±0.1	1.2±0.1	0.39	1.2±0.1	1.2±0.1	0.48

[a] Dual Energy X-ray Absorptiometery
[b] Mean±SD
[c] Bone Mineral Content
[d] Bone Mineral Density

When data were analyzed according to the gender, the results showed that, for the two females, fat mass and lean mass were not affected by the course of disease, however, BMD decreased significantly. Male subjects (n=3) lost both fat and lean mass, but preserved bone density.

The rate of fat and lean mass tissue changes was also calculated based on the tissue changes over the interval between T1 and T2. Table B.3.4 shows the results for all five subjects and for each gender separately.

Table B.3.4. The rate of tissue mass change (g/day) per day determined by DEXA in different genders

	Females (n=2)	Males (n=3)	P-value	Total (n=5)
Fat (g/day)	-8.7±35.6[a]	-46.3±36.0	0.16	-31.3±37.3
Lean (g/day)	49.9±50.4	-63.0±30.4	0.02	-17.8±70.2

[a] Mean ±SD

When the role of gender was not considered, the rate of fat change (loss) was almost twice the rate of lean mass change (loss) (-31.3 g/day vs –17.8 g/day). However, when data were compared by gender, females showed fat loss but preservation of lean mass whereas males showed losses of both fat and lean mass.

B.4. Nutritional intake

Table B.4.1 summarizes the dietary intake information from food records for each patient (n=9) at T1.

Table B.4.1. Individual baseline (T1) evaluation of dietary intake assessment in patients with advanced cancer (n=9)

Patient #	Energy (kcal/day)	Fat (g/day)	Fat (% of energy)	Carbohydrate (g/day)	CHO (% of energy)	Protein (g/day)	Protein (% of energy)	Energy (kcal/kg)	Protein (g/kg)	Sugar (g/day)	Calcium (mg/day)	Fiber (g/day)
1	2461.4	96.6	34.0	334.2	52.0	90.5	14.0	51.3	1.9	168.5	1525.2	14.2
2	3926.5	138.5	31.0	524.8	52.0	168.8	17.0	44.6	1.9	222.2	3139.5	34.1
3	1088.7	38.7	31.0	138.7	51.0	50.8	18.0	25.9	1.2	49.0	520.4	12.1
4	1822.4	65.5	32.0	238.9	52.0	76.0	16.0	38.4	1.6	114.0	1523.3	10.2
5	1995.7	68.5	31.0	277.8	55.0	73.0	14.0	25.6	0.9	141.6	1453.6	16.0
6	1084.1	33.1	27.0	170.1	61.0	33.1	12.0	17.9	0.5	127.8	145.4	4.2
7	2801.1	69.0	21.0	445.4	62.0	126.3	17.0	43.1	1.9	156.8	1445.2	34.3
8	1307.3	26.5	18.0	227.1	67.0	50.2	15.0	24.4	0.9	45.3	457.2	25.5
9	1422.2	64.1	39.0	182.0	49.0	43.9	12.0	21.7	0.7	91.5	928.3	23.2

The mean and SD of all variables in dietary intake assessment is shown in Table B.4.2.

Table B.4.2. Average of baseline (T1) dietary intake assessment in all the patients with advanced cancer (n=9)

Variable	Mean	SD	Range
Energy (kcal/day)	1989.9	940.7	1084.1-3926.5
Fat (g/day)	66.7	34.6	26.5-138.5
Fat (% of energy)	29.3	6.5	18.0-39.0
Carbohydrate (g/day)	282.1	130.5	138.7-524.8
CHO (% of energy)	55.6	6.2	49.0-67.0
Protein (g/day)	79.2	43.9	33.1-168.8
Protein (% of energy)	15.0	2.2	12.0-18.0
Energy (kcal/kg)	32.5	11.9	17.9-51.3
Protein (g/kg)	1.3	0.6	0.5-1.9
Sugar (g/day)	124.1	57.0	45.3-222.2
Calcium (mg/day)	1237.6	886.2	145.4-3139.5
Fiber (g/day)	19.4	10.5	4.2-34.3

Four patients completed two food records, each at the time of the DEXA scan. Students' paired-t test was conducted to examine the dietary difference between T1 and T2. (Table B.4.3.)

Table B.4.3. Dietary intake comparison between T1 and T2 in patients with advanced cancer (n=4)

Variable	T1	T2	P-value
Energy (kcal/day)	1943.3±817.8[a]	1947.5±348.6	0.50
Fat (g/day)	67.1±23.8	62.6±8.3	0.34
Fat (% of energy)	31.3±7.6	29.0±5.3	0.25
Carbohydrate (g/day)	275.1±141.1	291.2±73.8	0.38
CHO (% of energy)	53.5±5.8	57.8±7.4	0.22
Protein (g/day)	77.9±38.3	66.1±19.3	0.20
Protein (% of energy)	15.3±2.8	13.3±3.2	0.00*
Energy (kcal/kg)	35.4±13.9	36.4±4.6	0.42
Protein (g/kg)	1.4±0.6	1.3±0.4	0.25
Sugar (g/day)	116.5±56.3	114.8±42.6	0.46
Calcium (mg/day)	1104.8±470.9	1360.0±538.5	0.31
Fiber (g/day)	20.9±10.1	15.9±11.1	0.20

[a] Mean ±SD

High values for SD can be explained by a large range of energy intake between individuals. The results showed that only protein as a percentage of total energy was significantly lower at T2 compared to T1.

When gender difference was evaluated, the data did not show significant difference between dietary intake for males (n=2) and females (n=2). Also, there was no

significant difference for dietary intake between T1 and T2 for both male and female subjects.

C. Discussion

This case series study was completed to provide information regarding body composition changes over time, and to investigate the possible relationship between these changes and dietary intake in cancer patients with solid tumours. The findings of this study are categorized in three sections: Body composition change, dietary intake, and the relationship of both.

Body composition changes

Assessment of body composition was conducted using a DEXA scan. Over the period of four-six weeks, body composition change was different between subjects. When the first DEXA scan of all the subjects (n=9) was analyzed, comparison of the data revealed that there was a noticeable difference between males and females. Men represented more muscle mass and fat than females.

These finding are consistent with the results of other studies conducted in healthy subjects. In one study conducted by Abe e al. (18), the effect of gender on skeletal muscle was assessed in twenty healthy Japanese students using MRI. These researchers concluded that men had a greater total and regional skeletal mass than female subjects. The same finding has been reported by other studies (19). Therefore, one of the findings of this pilot study was that advanced cancer does not change the natural gender differences of body composition.

Five patients completed two DEXA scans over 4-6 week period. When the body composition of these patients was analyzed at T1 and T2, the results indicated that only fat mass was reduced over this short time from a mean of 9.9 kg to a mean of 8.4 kg (P value=0.06). Although the P-value did not meet the requirement value, it was very close to the significant level of < 0.05. In addition, BMC showed a noticeable trend in reduction at T2 compared to T1 (P value= 0.09).

Despite a very small sample size, the course of body composition change in male and female subjects was assessed. The results showed significantly reduced lean mass (p

value= 0.04) and a trend in decreased fat mass (p value=0.07) in male subjects. In contrast, neither fat nor lean mass change was significantly reduced in female subjects (p value of 0.34 and 0.18 respectively). However, females experienced a loss of bone mineral density (BMD) over this short period of time (p value= 0.01). A similar observation was derived from a study conducted by Harvie et al. (20). In their study, changes in body composition of male and female subjects with advanced non-small lung cancer (NSLC) were investigated before and after chemotherapy. This study showed that males tended to lose more lean mass compared to females. The etiology of this gender difference is not fully understood. The differences seen in the longitudinal body composition changes between male and female cancer patients may be explained by the original body structure. As it has been explained previously, males have greater mass of lean and fat mass compared to females. Therefore, the percentage of tissue loss is greater in males compared to females.

Measuring the rate of fat and lean mass wasting was one of the objectives of this study. To meet this purpose, the rates of change for both tissues were calculated (n=5). In general, males lost approximately 49 grams of fat and 63 grams of lean mass every day, whereas female lost 8 grams of fat but gained 49 grams of lean each day. Regardless of gender influences on body composition changes, the overall rate of loss is approximately 31 grams for fat and 17 grams for lean mass per day.

Findings of body composition changes in advanced cancer patients, using DEXA scan, can be summarized as following:

1-Gender differences in body composition in this small sample of advanced cancer patients were similar to individuals without cancer at baseline.

2-Over time, males showed greater wasting in both fat and lean mass compared to females.

3-Regardless of gender, in unselected cancer patients, fat is wasted faster than lean. However, if gender is considered in the measurement, male patients lose lean mass faster than fat mass.

Dietary intake

Three-day food records were used to assess energy and nutrient intake in participants. At the beginning of the study (T1), nine subjects provided food records, which were analyzed using Food Processor®, ESHA Research, Salem, OR. The results indicated that the mean energy intake by unselected cancer patients was 1990 kcal/day and the mean for protein intake was 79 g/d. Expressed in relation to body weight, the mean energy intake was 32.5 kcal/kgBW/d (SD=10) and for protein intake was 1.3±0.6 g/kgBW/d. Except for two patients who actually gained weight compared to pre-illness weight, seven patients lost ≥ 10% of their weight in six months prior to the enrolment to the study. High metabolism and the effect of mediators and cytokines in cancer patients are possible explanations. Lower energy and nutrient intake seen in the cancer population is a result of several factors such as poor appetite, vomiting, malabsorption, altered taste, and restricted mobility.

For four subjects who completed two dietary records, daily energy and protein intake were not significantly different at T2 compared to T1. However, the percentage of energy provided by protein was significantly lower at T2 (p value =0.00). Findings of nutritional intake in cancer patients can be summarized as following:

1- The average of daily energy and protein intake in this study population are similar to the average of energy and protein intake observed in the general population.
2- The wasting phenomenon associated with malignant disease could be related to low energy and protein intake.
3- The percentage of energy provided by protein was significantly lower at T2 (p value =0.00.

Food intake and body composition

Anorexia and malnutrition are common features of cachexia. It has been shown that the effect of nutritional interventions on body composition mainly is to increase fat but least effect on restoring lean mass (21;22). Due to the small sample size, and short interval between T1 and T2, this study failed to show any relation between nutrients

intake and body composition changes. However based on the previous studies, it is suggested that introducing more protein and amino acids to cancer patients, especially in combination with exercise, may retard the process of muscle erosion (23).

D. Limitations of the study

The target population for this study was palliative patients admitted to the Pain and Symptom Clinic and advanced cancer patients who were visited at the outpatient clinics located at CCI. Subject recruitment was limited by two main obstacles:

1- Palliative patients were approached at the Pain and Symptom Clinic, where they were managed for multiple physical and psychological complications such as pain, nausea, vomiting, anorexia, lack of energy and depression. At this point, the majority of these patients were not interested in participating in research. Among those who agreed to participate, a significant percentage of people had a deterioration in their health and they were subsequently unable to participate. From nine patients who enrolled in the study, one patient withdrew from the study after the first step and four patients died before completing the study. Therefore, the nature and progression of the disease was an important limiting factor.

2- Patients, who agreed to participate, were required to have two DEXA scans. Unfortunately, this service was not available at the CCI. Thus, the patients had to go another location (Medical Imaging Consultants, College Plaza, Edmonton, AB) for the scan. Many of these patients relied on their caregivers to make an extra trip for the DEXA scan. Most of the patients and their caregivers were reluctant to make such an effort.

E. Recommendation

This study was designed to follow body composition changes in advanced cancer patients over time. DEXA is one of the most accurate and reliable means to measure body composition. Due to the limitations of subject recruitment, it is suggested that advanced

cancer patients who are expected to have a longer survival be enrolled in future studies. In addition, if all facilities required for the study could be in the same location, this would encourage more patients to participate.

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Chapter Five

General discussion and conclusion

Cachexia is a very common complication of cancer, which can be used as a prognostic factor for survival in cancer. Preventing/delaying the wasting phenomenon in cancer improves the quality of life and survival. To achieve this goal, two questions should be answered:

1-Why wasting occurs: which requires understanding of the underlying causes of cachexia. There are many variables that influence body composition changes. Some of these factors are modifiable whereas some are not. Dietary intake, and exercise are those factors that can be controlled. In contrast, factors such as age and gender are not influence by interventions.

2- How it happens: which demands studying body composition in cancer population.

Different studies use different techniques to assess body composition. Some of these methods such as weight, skinfold thickness, are feasible and easily available. Clinical anorexia-cachexia research cannot discriminate fat or lean loss at all. It is also possible that muscle may be lost and a corresponding amount of fat gained and changes of these nature could be evolving within a constant body weight. The sometimes very large changes in visceral adipose tissue are not detected by skin fold analysis in conventional anthropometry. In addition, these methods may not be considered as sensitive and reliable as the advanced methods, such as DEXA, CT, and MRI, which are more expensive and are not available in most of the clinical cancer centres.

Using these imaging techniques enables researchers to measure different component tissues directly. However there is a price for this advantage. These methods work based on the action of x-ray radiation. Comparison between DEXA and CT/MRI reveals that DEXA involves less radiation exposure while CT/MRI scan allows measuring any particular tissue, at regional or total body level, precisely. In all cancer centres, patients are frequently required to have CT scans for diagnostic purposes.

Therefore, using the previously taken CT scans for research minimizes the cost and the radiation hazards.

In our research, we used CT scans and DEXA to measure body composition. The overall results of this study indicated that body composition change in the cancer population does not follow a unique pattern for all patients and each patient behaves differently. Our data make it possible to speculate on some of the important limitations of simply following body weight changes or conventional anthropometry in this patient population. Tissue gain or loss can clearly be composed of exclusively muscle, fat, or some fraction of both.

In this study, among lung cancer patients, the majority lost muscle (73%). Adipose tissue is another tissue that is wasted in progressive cancer, but the prevalence of adipose tissue loss (49-56%) is not as common as losing muscle. The results also showed that wasting adipose tissue is mainly from visceral depots rather than subcutaneous stores. Although cachexia is very common in cancer, our data showed that there is a subgroup of lung cancer patients, who tended to maintain or gain muscle (27%) or adipose tissue (44-51%). The results of this special group of cancer patients may be due to extra-calorie intake and decreased activity, which increases adipose tissue; or the effect of interventions such as exercise and androgen therapy, which improves muscle volume. However, these factors were not assessed and new prospective studies are required to evaluate associations between tissue changes and potentially causal factors.

When the role of gender and age was taken into account, both studies showed that males lost muscle and adipose tissue faster than females. With respect to age, the rate of visceral adipose tissue wasting was significantly higher in older patients. Although the younger group lost muscle faster, the rate of muscle wasting was not statistically significant.

The CT scan study only assessed regional body composition. Whether regional assessment represents whole body composition changes, requires future research. Changes in different regions should be compared with each other and with total body composition changes. Also, in the CT scan study, only lung cancer patients were evaluated. Further investigations are necessary to evaluate tissue change in other types of cancer.

Appendix A

Anatomical Background

1.1. Muscle group (1)

In all the CT images, analyzed in this study, four different muscles were investigated including Rectus abdominis, Oblique and transverse abdominus, Psoas major, and Para-spinal muscles. Each muscle has a different function and anatomy, which may result in different behaviour during cachexia. The goal was to study the pattern of wasting in each group.

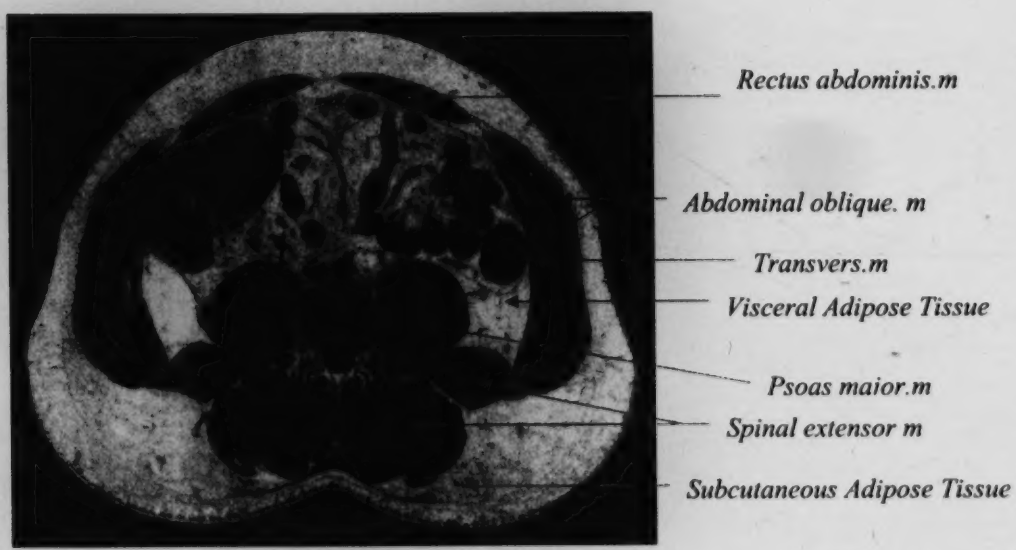
The Para-spinal muscles are a group of functional muscles that cover the anterior and posterior aspects of the spine. The Para-spinal muscles are responsible for initiating and controlling the vertebral column movement and trunk posture. This group is divided into two major subgroups of back extensors and flexors. The back extensor muscle group is composed of *erector spinae* and *multifidus*, and *Quadratus lumborum* muscles. While the function of the first two muscles is to extend the back against the force of gravity, the *quadratus lumborum* fixes the last rib (T12) during respiration. The main flexor muscle is the *psoas* muscle.

Anatomically, the erector spinae muscle originates from the iliac crest, sacrum, transverse and spinous processes of vertebrae and supraspinal ligament and inserts into angles of the ribs, transverse and spinous processes of the vertebrae, and the posterior surface of skull. Multifidus is another back extensor, which originates from the sacrum and transverse processes of C3-L5, and inserts into spinous processes of 2-4 vertebral levels superior to their origin. The Psoas muscle, the main back flexor is a fusiform shape muscle, which starts from the vertebrae bodies of T12 to L5, vertebral transverse processes of L1 to L5, and intervertebral discs of T12 to L5 and inserts into the medial surface of the lesser trochanter of the femur. The Psoas major's role is to flex and medially rotate at the hip joint, flex the vertebral column anteriorly, and flex the lumbar vertebral column laterally.

The Rectus abdominis is a long flat muscle, which extends along the whole length of the front of the abdomen, and is separated from its pair on the opposite side by the

linea alba. It is much broader, but thinner, above than below. The function of this muscle depends on which part of the muscle contracts. Contraction of the lower part causes vertebral column flexion and thorax depression. If the upper part of the muscle contracts, it flexes the pelvis upon the vertebral column.

Three flat muscles including internal oblique abdominal muscle, external oblique abdominal muscle, and transversus abdominal muscle are situated on the lateral and anterior part of the abdomen. Transverses abdominal muscle is the most internal of the flat muscles of the abdomen, being placed immediately beneath the internal oblique. The Internal muscle is smaller and thinner than the External muscle. The External oblique is the largest and the most superficial of the three flat muscles in this region. It is broad, thin, and irregularly quadrilateral, with its muscular portion occupying the side, and its aponeurosis occupying the anterior wall of the abdomen.



All mammalian skeletal muscle contains three distinct muscle fibre types (2;3). Type I or slow twitch fibres refer to those fibres with low ATPase activity, and prolonged twitch duration. In muscles which are involved in sustained isometric contraction, the type I is a predominant type of fibre to contract the muscle more economically. These fibres utilise less ATP to contract the same duration and the same force compared to the

other two types of muscle fibres. Therefore, less inhibitory metabolic end-products, which are significant factors in muscle fatigability, will develop (4;5).

In contrast, type IIA and IIB muscle fibres are those with fast twitch, shorter twitch duration and higher ATPase activity. These types of muscles are involved in more dynamic muscle functions such as the extensor digitorum lanugos (EDL). Few studies have investigated the composition of fibre type in the Para-spinal muscle. Mannion et al. reported that the main component of the erector spinae muscle group is type I muscle fiber, which is well suited for maintaining posture and extending the back to oppose the force of gravity (6).

The composition of muscle fibres in skeletal muscle is mainly influenced by genetic factors and sex. However, over time, other factors such as age, exercise, and disease can alter the proportion of different muscle fibre types within the skeletal muscle (7-12).

1.2. Adipose tissue

Adipose tissue is one of the largest body compartments with various biological functions including energy storage, mechanical cushioning, hormone release, and thermogenesis. In assessing body composition, a distinction should be made between fat and adipose tissue. Although these two terms are used interchangeably, fat and AT have different metabolic functions and compositions.

Fat is a triglyceride form of lipid, which is the major component of AT (13). Although its main depot is in AT, it is found naturally in other tissues such as skeletal muscle. In skeletal muscle, TG can be detected by magnetic resonance spectroscopy as an intermyocellular lipid (14). In some pathological conditions such as insulin resistance and muscular dystrophy the fat content of tissues other than AT, increases (15).

Adipose tissue can be classified according to its biological function (Brown, White, Bone marrow, and Mammary gland) or anatomical location. The traditional approach classifies adipose tissue based on the anatomical location such as subcutaneous AT and internal adipose tissue (visceral, non-visceral AT). Further classification is applied to both visceral (retro and intra-pritoneal) and non-visceral (paravertebral, intramuscular, perimuscular, and bone marrow AT) (16-18). AT, which surrounds an

organ is specifically named for that organ. These organs are mostly located in the retro-peritoneal area. Peri-renal, peri-pancreatic and peri-aortic AT are examples. Subcutaneous adipose tissue is the adipose layer between the dermis and the aponeuroses and fasciae of the muscles. Interstitial AT is infiltrated tightly between cells. With the aid of new technology such as CT and MRI, it can be detected, especially in skeletal muscles.

The importance of lipid as an energy substrate for skeletal muscle has been recognized for many years (19). The metabolic fate of fatty acids taken up by skeletal muscle is either oxidation for energy production, or esterification into triglycerides or phospholipids. In several studies non-invasive approaches such as CT and MRI have been used to study the muscle composition. Detection of fat deposition in either intermuscular or intra muscular form is challenging. In many organs adipocytes are scattered and difficult to detect by imaging techniques. Lipid has a low (negative) attenuation value in CT and MRI images, thus a low density value in muscle would be consistent with increased fat content within the muscle. Therefore, the precise approach to detect muscular change in adipose tissue is to rely on the density of the tissue.

It has been shown in some circumstances such as muscular dystrophy (Duchenne myodystrophy), poliomielitis, obesity, and AIDS, skeletal muscle decreases and adipose tissue increases (20-25). However, the mechanism for this tissue composition change is not fully understood.

No study has been found, which evaluates the changes in muscle composition in cancer patients. Thus, this present study was an investigation of this question.

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Appendix C

Appendix D

THREE-DAY DIETARY INTAKE RECORD

Name/ID: _____

Phone Number: _____

Date of Birth: _____ _____ _____
(Day) (Month) (Year)

Record Dates: _____ _____ _____ _____ _____ _____
(Day) (Month) (Day) (Month) (Day) (Month)

University of Alberta
Department of Agricultural, Food and Nutritional Science

INSTRUCTIONS FOR RECORDING DAILY FOOD INTAKE

The purpose of this study is to discover everything you eat and drink during a three-day period. It is important to record ALL foods and beverages – whether it is a full course meal at home or a quick can of pop at school/work. Before you start recording your intake, please read the following instructions and the Sample Day.

The Three-Day Dietary Intake Record has a separate section for every day (see Day 1, Day 2, Day 3 on top each page). Each day is broken up into 6 eating times:

1. Morning meal
2. Midmorning snack
3. Midday meal
4. Afternoon snack
5. Evening meal
6. Evening snack

It is a good idea to carry your Dietary Intake Record book with you and record your entries as soon after eating as possible. Foods and beverages consumed away from home – at a friend's house, at the mall, at a restaurant- are just as important as those eaten at home. Please include the following information on your food record:

1. **FOOD AND BEVERAGE ITEMS** Column: Enter all foods and beverages consumed at the meal or snack time. Please record the specific type of food (for example: *WHOLE WHEAT* bread, *FROSTED FLAKES* cereal). In the same column, record all toppings or items added at the time of eating (for example: sugar, syrup, jam, butter, mayonnaise, gravy, milk, salt, etc.). For combination foods, please include detailed information on each item. For example: If you had a tuna sandwich, you would list the following foods and include detailed information for each of them: white bread, mayonnaise,

3. **NUMBER OF UNITS** Column: In this area, record the number of units consumed. Include the amount of the food or beverage item and the amount of any topping or items added.

4. **UNIT OF MEASURE** Column: For every item consumed, enter the unit of measure you are using for this item. For example: enter the word "cup", "grams", "piece", "ounce", "number", "teaspoon", or "tablespoon". Enter a unit of measure not only for the menu item, but for toppings or items added as well. Each entry must have its own unit of measure. Use measuring cups and spoons whenever possible.

Fill in the blanks on the bottom of each record. Please list any vitamin or mineral supplements and/or herbal products taken, including quantities and detailed label information, if possible. Indicate the time of your meal or snack and where it was eaten (for example: at home, at a restaurant, in class). If you ate more than one snack between two meals, please indicate the time of each snack. If you did not eat a meal or snack, please place a check mark (✓) in the space provided on the bottom of the page, so that we do not think you forgot to record it.

Daily check: in the evening, after you have recorded everything for the day, go back over your entries to make sure you have included as much detail as possible for each item. Also check that the blanks are completed on the bottom of the page.

All foods and beverages you consume every day are important and your Dietary Intake Record should be as

celery, solid white tuna, salt.

2. **DESCRIPTION OF ITEM** Column: For every food or beverage item listed, include the following (if applicable):
   - **Brand**: *MIRACLE WHIP* mayonnaise, *PIZZA HUT DEEP DISH* pizza, *OREO* cookie
   - **Type of flavour**: *BLUEBERRY* muffins, *STRAWBERRY* yogurt
   - **Method of cooking**: *FRIED, BAKED, BBQ'D, HOMEMADE*
   - **All other relevant information included on food label**: *LOW FAT* ranch salad dressing, *28% M.F. (MILK FAT)* cheddar cheese, *LEAN* Ground Beef

accurate as possible. It should also reflect the way you usually eat. Please do not change your normal eating habits for the 3 days you are recording your food intake. Your honesty is crucial to the success of this research study. We have provided a page at the back of your food record for you to include any additional information that will help us interpret your diet. Recipes and information from labels are particularly helpful.

Thank you for your participation and cooperation with this study. Please look closely at the Sample Day before beginning your Dietary Intake Record. **If you have any questions please phone:**

_____

_____

Sample Meal

Food and Beverage Items	Description Of Item	No. Of Units	Unit Of Measure
Enter all foods and beverages consumed. For combination foods, please include detailed information on each item.	Include a detailed description of each food and drink item consumed including: - Brand name - Flavour - Method of cooking - All other relevant information on food/drink label	Enter number of units	Enter unit of measure: for example: cup, grams, ounce, piece, teaspoon, tablespoon
Spaghetti with tomato/meat sauce:			
Pasta	Spaghetti, cooked	2	Cup
Tomato sauce	Hunt's canned sauce, roasted garlic flavour	1	Cup
Meat balls	Made with extra lean ground beef	5	Number (1 oz/ball)
Parmesan cheese, grated	Kraft, 30% Milk Fat (M.F.)	1	Tablespoon
Garlic Bread:			
Italian Bread	Toasted	3	Piece (large slice)
Garlic Butter		3	Teaspoon
Caesar salad:			
Lettuce	Romaine	1	Cup
Croutons	Safeway brand, garlic flavor	2	Tablespoon
Bacon bits	Simulated flavour, No Name Brand	2	Tablespoon
Caesar salad dressing	Kraft, Fat free	2	Tablespoon
Milk	1%	1	Cup

Tiramisu	Sarah Lee	1	Slice
Coffee	Black	1	Cup

Vitamin/Mineral Supplements or Herbal Products taken:_____

_____

_____

Fill in blanks: Time of meal/snack: 6:00 pm **Location meal/snack was consumed:** at home

Please CHECK (✓) if you did not eat or drink at this meal or snack time:_____

Day 1 - Morning Meal

Food and Beverage Items	DESCRIPTION OF ITEM	NO. OF UNITS	UNIT OF MEASURE
Enter all foods and beverages consumed. For combination foods, please include detailed information on each item.	Include a detailed description of each food and drink item consumed including: - Brand name - Flavour - Method of cooking - All other relevant information on food/drink label	Enter number of units	Enter unit of measure: for example: cup, grams, ounce, piece, teaspoon, tablespoon

Vitamin/Mineral Supplements or Herbal Products taken:_____

_____

_____

Fill in blanks: Time of meal/snack:_____ **Location meal/snack was consumed:**_____

Please CHECK (✓) if you did not eat or drink at this meal or snack time:_____

Day 1 - Mid-Morning Snack

Food and Beverage Items	DESCRIPTION OF ITEM	NO. OF UNITS	UNIT OF MEASURE
Enter all foods and beverages consumed. For combination foods, please include detailed information on each item.	Include a detailed description of each food and drink item consumed including: - Brand name - Flavour - Method of cooking - All other relevant information on food/drink label	Enter number of units	Enter unit of measure: for example: cup, grams, ounce, piece, teaspoon, tablespoon

Vitamin/Mineral Supplements or Herbal Products taken:_____

_____

_____

Fill in blanks: Time of meal/snack:_____ **Location meal/snack was consumed:**_____

Please CHECK (✓) if you did not eat or drink at this meal or snack time:_____

Day 1 - Midday Meal

Food and Beverage Items	DESCRIPTION OF ITEM	NO. OF UNITS	UNIT OF MEASURE
Enter all foods and beverages consumed. For combination foods, please include detailed information on each item.	Include a detailed description of each food and drink item consumed including: - Brand name - Flavour - Method of cooking - All other relevant information on food/drink label	Enter number of units	Enter unit of measure: for example: cup, grams, ounce, piece, teaspoon, tablespoon

Vitamin/Mineral Supplements or Herbal Products taken:_____

_____

_____

Fill in blanks: Time of meal/snack:_____ **Location meal/snack was consumed:**_____

Please CHECK (✓) if you did not eat or drink at this meal or snack time:_____

Day 1 - Afternoon Snack

Food and Beverage Items	DESCRIPTION OF ITEM	NO. OF UNITS	UNIT OF MEASURE
Enter all foods and beverages consumed. For combination foods, please include detailed information on each item.	Include a detailed description of each food and drink item consumed including: - Brand name - Flavour - Method of cooking - All other relevant information on food/drink label	Enter number of units	Enter unit of measure: for example: cup, grams, ounce, piece, teaspoon, tablespoon

Vitamin/Mineral Supplements or Herbal Products taken:_____

_____

_____

Fill in blanks: Time of meal/snack:_____ **Location meal/snack was consumed:**_____

Please CHECK (✓) if you did not eat or drink at this meal or snack time:_____

Day 1 - Evening Meal

Food and Beverage Items	DESCRIPTION OF ITEM	NO. OF UNITS	UNIT OF MEASURE
Enter all foods and beverages consumed. For combination foods, please include detailed information on each item.	Include a detailed description of each food and drink item consumed including: - Brand name - Flavour - Method of cooking - All other relevant information on food/drink label	Enter number of units	Enter unit of measure: for example: cup, grams, ounce, piece, teaspoon, tablespoon

Vitamin/Mineral Supplements or Herbal Products taken:_____

_____

_____

Fill in blanks: Time of meal/snack:_____ **Location meal/snack was consumed:**_____

Please CHECK (✓) if you did not eat or drink at this meal or snack time:_____

Day 1 - Evening Snack

Food and Beverage Items	DESCRIPTION OF ITEM	NO. OF UNITS	UNIT OF MEASURE
Enter all foods and beverages consumed. For combination foods, please include detailed information on each item.	Include a detailed description of each food and drink item consumed including: - Brand name - Flavour - Method of cooking - All other relevant information on food/drink label	Enter number of units	Enter unit of measure: for example: cup, grams, ounce, piece, teaspoon, tablespoon

Vitamin/Mineral Supplements or Herbal Products taken:_____

_____

_____

Fill in blanks: Time of meal/snack:_____ **Location meal/snack was consumed:**_____

Please CHECK (✓) if you did not eat or drink at this meal or snack time:_____